

**QUANTITATIVE MICROBIAL RISK ASSESSMENT FOR *LISTERIA*
MONOCYTOGENES ON FRESH-CUT LETTUCE AND FRESH-CUT
CANTALOUPE**

A Thesis

by

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ABSTRACT

The increase in foodborne illness outbreaks associated with fresh and fresh-cut produce can be attributed to ineffectiveness of current handling practices. This study describes the change on concentration of population of *Listeria monocytogenes* in two popular fresh-cut produces, romaine lettuce, and cantaloupe, from farm to table.

Listeria innocua was used as a surrogate for *L. monocytogenes* to experimentally evaluate the effectiveness of washing treatments (water and chlorine) and develop growth curves under different storage temperatures (between 5 and 36°C). The findings confirm that both washing treatments were significantly more effective ($p < 0.05$) on reducing *L. innocua* concentration in fresh-cut romaine lettuce than in cantaloupe. For instance, chlorinated water washing reduced *L. innocua* population by 0.98 log on fresh-cut romaine lettuce compared to just 0.57 log on cantaloupe rind. Furthermore, the experimental data on *L. innocua* were used to test three predictive models to describe the growth of *L. monocytogenes* in both produce. All models (Baranyi and Roberts, Gompertz, and Logistic) provided good fit of the data. However, compared to the Baranyi and Roberts model, both Gompertz and Logistic models overestimated the growth rate at temperatures of 10°C and above. Results demonstrated that these models may be used to estimate the growth in fresh-cut produce during distribution, storage or at the market, and potential growth at a consumer level.

Several scenarios were created to evaluate the impact of decontamination treatments, occurrence of cross-contamination, and temperature abuse on the population of *L. monocytogenes*. In general, expected annual listeriosis cases associated with fresh-

cut cantaloupe were higher (around 17) than with fresh-cut romaine lettuce (<1). The time of consumption of the produce was the biggest issue regarding to ensuring the safety of the fresh-cut produce. Occurrence of temperature abuse and cross-contamination also increased the risk of listeriosis in both products. Among the intervention steps, irradiation treatment was the most effective, with 99.99% reduction on the expected number of annual cases of listeriosis for both produce.

DEDICATION

To my beautiful spouse and daughter, Nihal and Defne Guzel,

To my parents, Ali Osman, and Rukiye Guzel

For their unconditional love. Thank you.

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CHAPTER I

INTRODUCTION

The demand for fresh and minimally processed products has increased in recent years due to health trends and the technological advancements in food packaging. However, the number of foodborne illnesses related to this food commodity has risen gradually with the increase in demand (Beuchat, 2002; Mukherjee et al., 2006; Hoelzer et al., 2012a). Among the fresh produce, green leafy vegetables raise the most concern regarding microbiological hazards. Indeed, they have been connected to multiple outbreaks of foodborne disease (FAO/WHO, 2008).

Surveillance of fresh products and outbreak reports show that *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* are some of the most important pathogens associated with this commodity (Warriner et al., 2009; Franz et al., 2010; Tromp, 2010). According to the Center for Disease Control and Prevention (CDC) reports, 131 fresh produce related outbreaks occurred between 1996 and 2010, resulting in 1382 hospitalizations and 34 deaths (USFDA, 2013b). Twenty-two percent of the foodborne illnesses between 1998 and 2008 were linked to leafy vegetables, and eleven percent was linked to fruits (CDC, 2013a). Primarily, lettuce was the most aforementioned commodity (Delaquis et al., 2007; Adavi, 2011).

In the United States, 34 outbreaks related to melons occurred between 1973 and 2011 (Danyluk et al., 2014). Moreover, in the last three years, two deadly outbreaks were linked to cantaloupe consumption. In 2011, a *L. monocytogenes* outbreak led to 146 illnesses and 33 deaths, one of the deadliest foodborne outbreaks in the last 25 years

(CDC, 2012b). Although *L. monocytogenes* is not a common foodborne pathogen for fresh produce, the lethality ratio of *L. monocytogenes* (18%) is higher than common pathogens like *Salmonella* (<1%) (Painter et al., 2013). Consequently, the presence of *L. monocytogenes* in fresh products is a main concern to producers and processors (Lianou and Sofos, 2007; Koseki et al., 2011; Sant`Ana et al., 2012a). Therefore, studies on prediction of the occurrence of this pathogen in a particular type of food product is critical to establish proper handling and distribution practices to avoid potential foodborne illness. These studies can be based on actual experiments or via predictive models.

When it is necessary to conduct experiments, *L. innocua* is often used as a surrogate for *L. monocytogenes*, and is preferred in laboratory experiments for several reasons. First, it is not pathogenic, so the health risks for laboratory personnel are minimal. In addition, *L. innocua* shows similar characteristics to *L. monocytogenes*, and sometimes is even referred to as a non-pathogenic variant of *L. monocytogenes* (Jay, 2004).

Predictive modeling can provide valuable information on the growth, decline, and survival of pathogens in foods during processing and storage (Whiting, 1995; Perez-Rodriguez and Valero, 2013), and dynamic models are useful to estimate the population dynamics of pathogens in food systems at time-varying temperature profiles (non-isothermal conditions) (Puerta-Gomez et al., 2013). Furthermore, predictive modeling is a useful and powerful tool that is necessary to estimate the growth of pathogens in quantitative microbiological risk assessment. Quantitative microbiological risk

assessment (QMRA) is a relatively new concept in predictive microbiology, and it is drawing more attention day by day. QMRA facilitates scientists' understanding of the impact of prevention strategies on the pathogen population in foods. Because the estimation of microbial risk naturally consists of variability and uncertainty, simulation methods such as Monte Carlo are generally used in QMRA (Danyluk and Schaffner, 2011).

Another benefit of QMRA is the evaluation of the effect of safety procedures on processing with risk assessment techniques that allow producers to estimate results before the product leaves the facility. Additionally, quantitative risk assessment methods are helpful to inform policy decisions that depend on the problem, the period, and the specific risk management questions to be addressed. These models are also useful to predict the microbiological shelf-life of perishable foods such as fresh-cut produce.

The main objective of this study was to generate a quantitative risk assessment model of human health risk involving contamination of fresh-cut cantaloupe and fresh-cut romaine lettuce with *Listeria monocytogenes*. The specific goals were:

(1) To use growth data on *L. innocua* to predict the growth of *L. monocytogenes* on fresh-cut romaine lettuce and fresh-cut cantaloupe as a function storage temperature, and to compare the response of *L. innocua* to washing treatments on both produce.

(2) To conduct quantitative risk assessments on the effect of intervention and handling steps to minimize the potential risk of contamination of the fresh-cut produce with *L. monocytogenes*.

CHAPTER II

LITERATURE REVIEW

2.1 Consumption of Romaine Lettuce and Cantaloupe in the U.S.

Romaine lettuce (*Lactuca sativa*, var *longifolia*), also called Cos lettuce (originated from a Greek island; Kos) is a leafy green vegetable that is mostly eaten raw. Lettuce is a good source of antioxidants, as well as vitamin A and C. Although it has a very long history and is very popular in Mediterranean countries, In the U.S romaine lettuce started gaining popularity only in the late 80's (de Vries, 1997; USDA, 2005).

According to the U.S. Department of Agriculture (USDA) data, per capita use of romaine lettuce was 0.33 kg in 1985, 1.27 kg in 1995, and 3.49 kg in 2009.

Correspondingly, while romaine lettuce consumption covered 2% of all lettuce consumption in 1985, this ratio increased to 27.4% in 2009 (USDA, 2011). This drastic increase in consumption can be linked to advances in food technology. Romaine lettuce was a member of raw agricultural commodities (RAC), which means it had to be washed properly before consumption. Today, romaine lettuce can be considered as a member of ready-to-eat (RTE) foods, which means it is pre-washed, and does not need further treatments at home. Lettuce is consumed by 40% of the U.S. population, and 85% of this population consumes lettuce as fresh (Hoelzer et al., 2012a). Considering the fact that romaine lettuce consumption covers 27.4% of total lettuce consumption, it can be concluded that romaine lettuce is consumed as a fresh leafy green by 9.31% of the U.S. population.

Cantaloupe (*Cucumis melo* L.) a popular member of melon family, is an important phytonutrient source (Castillo et al., 2009). Although melons are produced across the U.S., 80% of production is gathered in five states: California, Arizona, Georgia, Florida, and Texas. Cantaloupe is also good source of iron, potassium, fiber, antioxidants and vitamins A and C (Beaulieu and Lea, 2007). Per capita use of cantaloupe in the U.S. was 3.87 kg in 2010 (USDA, 2012). Cantaloupe is consumed by 3.03% of the U.S. population as a fresh fruit (Hoelzer et al., 2012a).

2.2 Foodborne Disease Outbreaks Associated with Fresh Produce

It is well known that bacteria, viruses, and protozoan cause foodborne disease outbreaks in fresh produce epidemiologically; however, bacterial origin foodborne disease outbreaks linked to fresh produce have been commonly reported (Olaimat and Holley, 2012). Since the 90's, There has been a sharp increase in the number of outbreaks linked to fresh produce in the U.S. While the ratio of outbreaks linked to fresh produce to total outbreaks was less than 1% in the 70's, the number increased to 6% in the 90's, and 13% in 2005 (Doyle and Erickson, 2008). A recent study suggests that fresh produce related outbreaks cover more than 40% of total outbreaks in the U.S. (Painter et al., 2013). Although there is no clear connection, the number of foodborne outbreaks linked to fresh produce has increased in the past three decades with the increased consumption (Sivapalasingam et al., 2004; Lynch et al., 2009; Vadlamudi et al., 2012). Beside the increased consumption of fresh produce, changes in pre and postharvest processing, distribution, and consumption patterns are believed have significant roles in this increase (Beuchat and Ryu, 1997; Burnett and Beuchat, 2001;

Prazak et al., 2002; Lianou and Sofos, 2007; Lynch et al., 2009; Painter et al., 2013). Moreover, the improvements in pathogen identification methods and the increase in size of susceptible population may also play a role on the increase in the outbreaks (Tauxe, 1997; Carrasco et al., 2010). Burnett and Beuchat (2001) reported that fresh produce, such as lettuce and cantaloupe had recently been linked to foodborne diseases.

Incidence of listeriosis outbreaks are less than other pathogenic diseases. 21 reported outbreaks linked to *L. monocytogenes* occurred between 1998 and 2008, whereas 870 *Salmonella enterica* and 206 *E. coli* outbreaks were reported in the same period (Painter et al., 2013). However, compared to other common pathogens, *L. monocytogenes* has a high mortality rate (Lianou and Sofos, 2007). It was estimated that *S. enterica* caused more than one million illnesses annually, but only 1.8% of the patients were hospitalized, and among the hospitalized 1.9% resulted in death, while 1% of the patients were hospitalized, and less than 1% of them resulted in death in illnesses caused by *E. coli*. On the other hand, it was estimated that *L. monocytogenes* caused around 1500 illnesses 91% of which were hospitalized, and about 18% of hospitalizations resulted in death annually (Painter et al., 2013). The first reported listeriosis outbreak was in 1979. Tuna and chicken salad with leafy greens contaminated by *L. monocytogenes* caused 20 hospitalizations and five deaths (Ho et al., 1986). In total, 12 listeriosis outbreaks linked to fresh produce consumption occurred worldwide. Seven of these outbreaks happened in the U.S. resulted in 189 hospitalizations and 40 deaths (Table 2.1). Recent listeriosis outbreak associated with cantaloupe was the deadliest foodborne outbreak in the last 25 years (Danyluk et al., 2014). With the

improvements in pathogen detection, sometimes outbreaks can be prevented by recalling the contaminated product.

Table 2.1. Outbreaks of listeriosis associated with fresh produce consumption in the U.S. (Adapted from Hoelzer et al., 2012b).

Year	Source	No. of cases	No. of hospitalizations	No. of fatalities
1979	Tuna fish and chicken salads with celery, lettuce, and tomatoes, cheese	20	20	5
	Frozen broccoli, cauliflower	7		
2001	Potato salad	56	1	0
2006	Taco/nacho salad	2	0	0
2010	Alfalfa sprouts	20	16	0
2010	Celery	10	10	5
2011	Cantaloupe melon	146	142	30
Total		261	189	40

However, even though recalls are very beneficial for public health, they are costly solutions. Romaine lettuce was recalled 15 times in the past three years, 12 of which were associated with *L. monocytogenes*. In the same time, cantaloupe was recalled

seven times, and *L. monocytogenes* was the reason for four of these recalls (USFDA, 2014).

2.3 Contamination of Cantaloupe and Romaine Lettuce

2.3.1 Contamination of Cantaloupe with *L. monocytogenes*

Cantaloupe can be contaminated with pathogens in the field, and during post-harvest applications. However, when intact, the outer rind works as a protective layer against bacteria, and limits the accumulation in fruit flesh. Several studies showed that even though the bacterial load on the rind was high, only a limited number of bacteria were found in the flesh. On the other hand, fissures, cuts, scar tissues, and ground spots promote the internalization of pathogens (Castillo et al., 2009). The ground spot is the connected area of fruit to surface, and the rind of that area is thin. In addition to structural defects, insects may help pathogens to contaminate the fruit. Caldwell et al. (2003) reported that *Salmonella* could be introduced to fruit rind by a nematode. Moreover, Richards and Beuchat (2004) showed that temperature differential and surface characteristics of cantaloupe affects internalization of pathogens. Internalization can even occur through intact rinds (Bowen et al., 2006). The mesocarp tissue is highly susceptible. As a result, if the rind is damaged, bacteria can infiltrate into the flesh very quickly. Chimbombi et al. (2013) reported that *Salmonella* might infiltrate the cantaloupe flesh in 10 hours at 23°C. Moreover, a bacterial population of 4.2 log CFU/g was observed in a 50 mm depth in 30 hours at 23°C. In postharvest practices, the main sources of contamination are washing/sanitizing step, worker activities during handling, and the equipment used in postharvest. Contamination can occur by direct contact or

cross-contamination (Castillo et al., 2009). Castillo et al. (2003) surveyed cantaloupes in the U.S. and Mexico. In Mexico, *Salmonella* and *E. coli* were isolated only in postharvest samples. In another microbiological survey conducted on cantaloupes, *Salmonella* were found in 16% of the samples taken from worker's hands. In the same study, it was determined that 20.6 % of packed cantaloupes were contaminated by *Salmonella* (Espinoza-Medina et al., 2006). Recently, Chen et al. (2013) reported that *L. monocytogenes* was isolated from 5 of 425 fresh-cut cantaloupe samples.

2.3.2 Contamination of Romaine Lettuce with *L. monocytogenes*

Like cantaloupe, romaine lettuce can be contaminated with water, workers, and equipment and transporting vehicles (FAO&WHO, 2008). Contamination can occur through direct contact or cross-contamination (Table 2.2).

Water quality is an important pre harvest factor due to contamination risk of large surface area of romaine lettuce (WHO/FAO, 2008). Brandl and Amundson (2008) determined that leaf age also affects the presence of pathogens in lettuce. Pathogens inoculated on young leaves growth significantly faster compared to older leaves. It is suggested that higher nitrogen and carbon content of young leaves promotes pathogen growth. In addition, cutting/shredding process helps bacteria to penetrate into the stomata by increasing handling, damaging the structure, and increasing the attachment surface (Jay, 2006). Takeuchi et al. (2000) found that *L. monocytogenes* attached the cut edge of lettuce more than surface. Aruscavage et al. (2008) determined that survival rate of *E. coli* O157:H7 on damaged lettuce leaves was higher than the healthy ones.

Table 2.2. Preharvest and postharvest contamination points of minimally processed fresh produce (Adapted from Harris et al., 2003).

Preharvest	Postharvest
Soil	Harvesting equipment
Irrigation water	Human handling
Inadequately composted manure	Air
Air	Wild and domestic animals
Wild and domestic animals	Transport containers
Human handling	Wash and rinse water
	Ice
	Packing, sorting and cutting process equipment
	Fruits, vegetables
	Improper storage
	Cross contamination
	Improper handling after wholesale
	Cooling water

2.4 *Listeria monocytogenes*

L. monocytogenes is a gram-positive, facultative anaerobic, non-spore forming, oxidase negative, catalase positive, small, rod-shaped bacterium (Farber and Peterkin, 1991). The genus of *Listeria* includes seven species, *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, *Listeria grayi*, *Listeria murrayi*, and *Listeria seeligeri*, all of which are widely distributed in nature. Although *L. monocytogenes* is the main pathogenic subspecies of *Listeria*, *L. ivanovii*, *L. seeligeri* also caused listeriosis very rarely (McLauchlin et al., 2004).

Listeriosis is a severe disease caused by *Listeria* genus. Almost all of human listeriosis cases are foodborne (Adak et al., 2002). Even though incidence of this disease

is a very small portion of all foodborne diseases, about 27% of foodborne disease deaths are linked to listeriosis (Mead et al., 1999). In addition, listeriosis has a very high (approximately 20%) mortality rate (Gellin and Broome, 1989; Farber and Peterkin, 1991). Listeriosis mostly occurs in infants, elderly, and pregnant women. Majority of listeriosis cases occur in the population groups who have an underlying condition, like immunosuppressed, AIDS, alcoholism, and diabetes (McLauclin et al., 2004). Since *L. monocytogenes* is widely distributed in nature, only way to control listeriosis is controlling *L. monocytogenes* contamination of food (Painter and Slutsker, 2007).

L. innocua is often used as a surrogate for *L. monocytogenes* in the experimental stage. Omary et al. (1993) used *L. innocua* to determine the growth of *L. monocytogenes* in shredded cabbage. Houtsma et al., (1994) used *L. innocua* to show the growth of *L. monocytogenes* in the presence of different sodium lactate and sodium chloride levels. Furthermore, Francis and O' Beirne, (1998) reported that *L. innocua* and *L. monocytogenes* show similar characteristics in lettuce under a range of conditions.

In another study, Geysen et al. (2005) used *L. innocua* for expressing the effect of super atmospheric oxygen and carbon dioxide on *Listeria monocytogenes* growth. Murphy et al. (2000) used *L. innocua* M1 for cooking method validation of *L. monocytogenes* in thermal processing. Behrsing et al. (2003) used *L. innocua* to show the survivability and growth of *L. monocytogenes* on cantaloupe. Rod et al. (2012) described the effect of cold atmospheric plasma on *L. monocytogenes* in ready-to-eat meat by using *L. innocua* as a surrogate. Omac et al. (2015) modeled the growth of *L.*

innocua and *L. monocytogenes* in different temperatures (5 to 36°C), and found that *L. innocua* and *L. monocytogenes* showed growth patterns on baby spinach.

Although *L. innocua* has been used as a surrogate in a number of studies, it does not necessarily show the same characteristics with *L. monocytogenes* in all instances (O'Bryan et al., 2006). Rodriguez et al. (2006) showed that *L. innocua* is not a suitable surrogate for *L. monocytogenes* under e-beam radiation. Friedly et al. (2008) found that under thermal conditions *L. innocua* M1 behaved differently than *L. monocytogenes* in hamburger patties. Hence, before using *L. innocua* as a surrogate in a new matrix, validation is required.

2.5 Survival and Growth of *Listeria monocytogenes*

The growth of *L. monocytogenes* on fresh produce is affected by several factors such as temperature, water activity, pH, and microbial competition. *L. monocytogenes* is a psychotropic pathogen and can grow under low temperatures (Warriner et al., 2009). Koseki and Isobe (2005) determined the minimum growth temperature of *L. monocytogenes* on lettuce as -4.26°C. In addition, *L. monocytogenes* can grow between pH 4.1 to 9.6 (Monsalve, 2008). *L. monocytogenes* and other pathogens survive and grow in fresh produce in several ways. The netted surface of cantaloupe may enhance the attachment ability of bacteria. Ukuku and Fett (2002) reported that *L. monocytogenes* can survive on the cantaloupe rind 15 days at 4°C, and the organism can grow within 4 hours at 20°C. Annous et al. (2005) observed biofilm formation on the rind surface of cantaloupe, two hours after inoculation with two different *Salmonella* isolates (*S. enterica* sv. Poona, *S. enterica* sv Michigan). Biofilms are groups of bacteria that attach

both to the surface and to themselves by producing polymeric materials. Attachment surface, presence of different bacteria, and strain difference may affect the formation of biofilms (Jay et al., 2006). Biofilm formation not only protects bacteria from harm but also may help bacteria to reach non-contaminated areas (Castillo et al., 2009).

Naturally existing microbiota in fresh products is believed to have an effect on the growth of *L. monocytogenes*. Initial microbial load present in lettuce is determined between 4 and 6 log CFU/g. (Carrasco et al., 2008). Carlin et al. (1996) reported that when endive leaves treated with 10 % hydrogen peroxide, *L. monocytogenes* grew very quickly on endive leaves because the number of native microorganisms on endive leaves decreased. Similarly, Ukuku et al. (2004) reported that *L. monocytogenes* attached to cantaloupe rind in greater numbers when native microflora is reduced. Francis and O'Beirne (1998) showed that varying the population of natural microbiota in shredded lettuce did not affect the growth of *L. innocua*, while the lactic acid bacteria (LAB) and *Enterobacter* spp. reduce the growth. It can be concluded that presence of specific competitive bacteria like LAB and *Enterobacter* spp. may play more important role than total initial population (Carrasco et al., 2008). Koseki and Isobe (2005a) also found that natural microbiota of iceberg lettuce has no effect on *L. monocytogenes*. Johnston et al., (2009) determined that the natural microbiota of fresh cut lettuce showed inhibitory effect on *E. coli* O157:H7. Acid production or antimicrobial peptides were associated with microbial antagonism.

2.6 Decontamination Methods for Fresh Produce

In 1998, the FDA released a guide that described the main reservoirs of pathogen contamination, methods for pathogen control, and methods to decrease the risk of pathogen contamination (USFDA, 1998). Most of the times, washing treatment is the only control point for fresh fruit and vegetables. Although a vast number of antimicrobial and chemical solutions were tested as a disinfection agent, chlorine is the most used component in the sanitation process. Chlorine is applied to wash water between 100 ppm and 200 ppm (Beuchat, 1998; WHO, 1998). Even though washing treatments are convenient for reducing the microbial population, microbiota of fresh vegetables cannot be eliminated by these treatments (Zhang and Farber, 1996; FAO/WHO, 2008; Carrasco et al., 2010). In addition, reusing washing water may cause cross contamination, and increase the microbial load of the product.

With current and widely used disinfection methods, microbial load of fresh produce can be reduced between 2 and 3 log units (Gil et al., 2009). However, as the pathogens are not removed, the growth will continue during shelf life. This problem may lead the manufacturers having to recall these kinds of products, and cost increases. In 2011 and 2012, seven recalls associated with romaine lettuce occurred. Four of these recalls related with potential *L. monocytogenes* contamination (USFDA, 2013c). As a result, manufacturers should be aware of the microbial shelf-life and the possible pathogens that may be present in the product. Lastly, manufacturers are in need of developed and improved disinfection methods.

2.6.1 Washing and Sanitizing Treatments

Among the chemical sanitizers, chlorine is the most widely used sanitizer in the industry. Acidic electrolyzed water, sodium hypochlorite, peroxyacetic acid, aqueous chlorine dioxide, water, and organic acid solutions (citric, acetic, or lactic acids) are some other disinfection agents that can be used in washing treatment step (James, 2006; Yuk et al., 2006). Rodgers et al. (2004) compared the effectiveness of several sanitizers including peroxyacetic acid, chlorinated trisodium phosphate, chlorine dioxide, and ozone on *L. monocytogenes* in whole and shredded lettuce. In shredded lettuce, it was found that peroxyacetic acid (80 ppm) caused 4.6 log reductions. Similarly, chlorinated trisodium phosphate (200 ppm chlorine) reduced the population by 4.6 logs. Chlorine dioxide was more effective than these two treatments, resulted in 4.7 log reduction, whereas ozone treatment was the most effective (3 ppm) reduced the *L. monocytogenes* population more than 5 logs. Exposure times (5 min) were same for all the treatments. For whole lettuce, all treatments were more effective compared to shredded lettuce, where *L. monocytogenes* population was reduced more than 5 logs. It is proposed that cutting/shredding process decreases the sanitizer effectiveness by creating more attachment surfaces to bacteria, and increasing the organic matter content in wash water (Rodgers et al., 2004).

In a similar study, effectiveness of chlorinated trisodium phosphate, ozone, peroxyacetic acid, and chlorine dioxide on *L. monocytogenes* on whole cantaloupe were evaluated. In 5 minutes exposure time, all sanitizers reduced the *L. monocytogenes* population more than 4.9 log CFU/g (Rodgers et al., 2004). In another study, Ukuku et

al. (2005) mixed H₂O₂ (1%), sodium lactate (1%), citric acid (0.5%), and nisin (25 µg/mg) and observed 3 to 4 log CFU/g reduction on *L. monocytogenes* population inoculated on cantaloupe rind. What is more, after that treatment *L. monocytogenes* was not detected on fresh cut pieces. In the same study, researchers also observed the effect of H₂O₂ (2.5%) alone. Researchers reported that treating cantaloupe rind with H₂O₂ (2.5%) alone was less effective than treating with cocktail. Also, *L. monocytogenes* was detected on fresh cut pieces when cantaloupe rind was treated with H₂O₂ (2.5%) (Ukuku et al., 2005). In a similar study, Ukuku and Fett (2002) treated *L. monocytogenes* inoculated cantaloupe rinds with H₂O₂ (5%) and 1000 ppm chlorine. *L. monocytogenes* was consistently absent on fresh-cut pieces when the rind was treated, and consistently found when the rind was untreated, or washed only with water. Palekar et al. (2004) made a similar observation; when cantaloupe rinds were treated with chlorine, the bacteria count inside the flesh was less than cantaloupes treated only with water. Mahmoud et al. (2008) treated whole cantaloupe with chlorine dioxide gas (5 mg l⁻¹) and achieved 3 log CFU reduction of *L. monocytogenes*.

Ozone is probably one of the most studied control methods. The effect of the ozone treatment on *L. monocytogenes* in lettuce has been studied extensively (Koseki et al., 2001; Rodgers et al., 2003; Koseki and Isobe, 2005; Yuk et al., 2006; Olmez and Akbas, 2008). Ozone (O₃) is a highly reactive oxidant showing inhibitory effect against not only wide range of bacteria, but also pesticides and chemical residues (Rodgers et al., 2003). In addition, unlike chlorine, temperature and pH has no influence on the effectiveness of ozone (Yuk et al., 2006; Olmez and Akbas, 2008). Furthermore, ozone

degrades spontaneously to oxygen, and leaves no residue on the product. Since ozone is a highly reactive compound, it can also affect the sensory quality of the product negatively. To minimize this effect, optimization of ozone treatment is necessary (Olmez and Akbas, 2009).

Several promising treatments have been developed in the last decades due to ineffectiveness of current disinfection methods. Cold atmospheric plasma is a new disinfection method that can efficiently reduce the pathogens in vegetables. Critzer et al. (2007) showed that cold atmospheric plasma reduce the *L. monocytogenes* population on lettuce by 1, 3 and 5 log CFU/25 cm² in 1,3, and 5 minute exposure times respectively. However, there are some downsides of this treatment. First, effect of the reactive compounds created by cold atmospheric plasma may also damage the product. Hence, this treatment may reduce shelf life. In addition, although atmospheric plasma is highly effective against the pathogens on product surface, it may not eliminate the internalized pathogens effectively. Lastly, feasibility of atmospheric plasma has not been tested industrially yet. Despite these drawbacks, atmospheric plasma is a new technology that is still being developed, and has not reached its full potential (Perni et al., 2008).

2.6.2 Irradiation

Ionizing radiation can be obtained with gamma rays, X-rays and electron beams. Unlike surface treatments, irradiation can penetrate the tissues, and eliminate the internalized bacteria (Gomes et al., 2011). Gomes et al. (2009) showed that when lettuce was treated with ionizing radiation up to 1 kGy it was possible to reduce the *E. coli* O157:H7 population by 3-4 logs. Although irradiation is an effective control measure

against the pathogens, it also affects the overall product quality when used in higher doses. Castell-Perez et al. (2004) reported that e-beam irradiation at higher than 1.5 kGy dose affects the quality attributes of fresh-cut cantaloupe negatively. Han et al. (2004) showed that irradiation affects the overall quality of whole romaine lettuce at doses 1.5 kGy and higher.

The effect of irradiation on bacteria is influenced by intrinsic and extrinsic factors. An example of intrinsic factors is the product composition. For example, required dose for 1 log reduction of *L. monocytogenes* was found around 0.5 kGy in meat products (Zhu et al., 2005). However, in lettuce the D₁₀ value was just 0.17 kGy (Mintier and Foley, 2005). Subgroups of food commodity can also affect the radiation sensitivity. Niemira (2003) observed the changes in radiation sensitivity of *L. monocytogenes* and *Salmonella* in four different lettuce types (Green leaf, Red leaf, Boston, and Iceberg). Although radiation sensitivity of *Salmonella* affected by lettuce type, the sensitivity of *L. monocytogenes* was stable. In addition to intrinsic factors, extrinsic factors influence the effectiveness of irradiation treatment. In fresh produce, the interior atmosphere of package, and temperature are some of the parameters that influence the radiation sensitivity. Niemira and Fan (2005) reported that produce and pathogen type, produce condition (whole, peeled, cut), and the atmosphere in the package have an impact on the efficiency of irradiation. On the other hand, Moreira et al. (2012) proved that handling, exposure, dose uniformity, and processing parameters are crucial factors for effectiveness of irradiation treatment. In that study, when does

uniformity ratio changed from 1.1 to 1.4, D₁₀ values of fresh products changed up to 53%.

Large size of cantaloupe, outer rind and penetration issues, make the treatment of whole cantaloupe with e-beam irradiation a challenge. Kim et al. (2010) reported that for 1 kGy dose on whole cantaloupe, 3.3 and 3.5 log CFU reduction of *Salmonella* was observed at 0.2 cm and 0.4 cm depths respectively. As a result, irradiation treatment should be carried out after packaging of fresh-cut pieces. *L. monocytogenes* has a radiation D₁₀ value (D₁₀ = 0.15 kGy) on fresh-cut cantaloupe (Rodriguez et al., 2006). Mahmoud (2012) showed that X-Ray irradiation with a dose of 1 kGy reduced the *L. monocytogenes* population by 4.1 log CFU 5cm⁻². Moreira et al. (2012) reported that D10 values for *E. coli* spp. and *Salmonella typhimurium* (LT2) on fresh-cut cantaloupe are 0.21 and 0.15 kGy respectively.

2.7 Predictive Microbiology

Microbial growth is the main reason of food spoilage and food poisoning. As a result, understanding the growth patterns and influential factors on growth is crucial (Peleg and Corradini, 2011). Some of these influential factors as; temperature, pH, water activity, antimicrobials, organic acids, competitive microbiota, sodium nitrite, and sodium chloride. Different models can be derived to predict the growth as a function time, temperature and commodity characteristics. These models can be helpful in determining the optimal process and storage conditions to control *L. monocytogenes* growth (Hoelzer et al., 2012a)

Predictive models can be divided into three groups; Survival/inactivation models, boundary models, and growth models (Perez-Rodriguez and Valero, 2013). Based on their development, predictive models can also be divided into three groups;

2.7.1 Primary Models

Marks (2008) defined the primary models as the description of population change as a function time under specific conditions. Perez-Rodriguez and Valero, (2013) indicated that primary models should explain the microbial growth accurately with the fewest of variables.

Monsalve (2008) characterized microbial growth curve by four main phases as follows: (1) the lag phase or the adaptation period described as an adjustment period throughout which bacterial cells adapt themselves to get advantage of the new environment and initiate exponential growth; (2) the exponential or logarithmic phase defined as the grow of microorganisms in their environment until they reach a maximum population level; (3) the stationary phase defined as the time when the growth rate of microorganisms equals the death rate of microorganisms; and (4) the death phase stated as the period when the microbial population starts to decrease because of reduced concentration of nutrients or physiological state of cells.

2.7.1.1 The Logistic Model

A three parameter logistic model was used to predict the growth of *L. monocytogenes* in fresh-cut cantaloupe (Fang et al., 2013). Fujikawa et al. (2004) reported that although bacterial growth curves are generally sigmoid on a semi-logarithmic plot, the logistic model generates a convex curve consisting of a

monotonously increasing portion and stabilizing one, without a lag phase at the initial period. Therefore, for fitting the bacterial growth data, the equation of logistic model was modified as follows (Chowdhury et al., 2007):

$$y(t) = C + (A/(1 + \exp(-B(t - M)))) \quad (2.1)$$

herein, C is the initial level of inoculation (log CFU/g); A represents the difference between the maximum and minimum growth values (log CFU/g); M is the time (hours) at which the slope of the sigmoidal growth reaches a maximum value; and B represents the maximum growth rate relative to the amount of growth at time M .

2.7.1.2 Gompertz Model

Isothermal microbial growth can be described with a sigmoidal function (Peleg and Corradini, 2011). Gompertz model is probably the most used empirical based sigmoidal function in predictive microbiology (Marks, 2008). The Gompertz model was used to describe the growth of *L. monocytogenes* in lettuce (Ding et al., 2010). The Gompertz growth model is given as follows (Gibson et al., 1988):

$$N(t) = C + A * \exp(-\exp(-B(t - M))) \quad (2.2)$$

herein, C is the value of the lower asymptote (log CFU/g); A is the asymptotic term (\log_{10}), M is the time at which the slope of the sigmoidal growth reaches a maximum value and the B is the maximum growth rate relative to the amount of growth at time.

2.7.1.3 Baranyi and Roberts Model

Baranyi and Roberts model was used in many studies regarding to fresh produce to estimate the growth of *L. monocytogenes* (Koseki and Isobe, 2005a; Ding et al., 2010; Puerta-Gomez et al., 2013a; Danyluk et al., 2014). In several studies, this dynamic

model was successfully implemented for a variety of growth conditions such as temperature, pH, and water activity. DMFit Excel Add-In software (Norwich, UK) is used to fit the model. Baranyi and Roberts (1994) described the differential equation that used in the Baranyi model as:

$$\frac{dx}{dt} = \alpha(t) * \mu_{max} * u(x) * x \quad (0 \leq t < \infty; 0 < x)$$

(2.3)

where, $\alpha(t)$ is a process of adjustment function (CFU/g); $u(x)$ is the indication of the inhibition function as it explains the transition of the growth curve to the stationary phase (CFU/g); μ_{max} is the maximum growth rate (h^{-1}).

The logarithm of the solution of Eq. (1), $y(t) = \ln(x(t))$, can be expressed as:

$$y(t) = y_o + \mu_{max} * F(t) - \ln\left(1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_o)}}\right)$$

(2.4)

$$F(t) = t + \frac{1}{v} \ln(e^{-vt} + e^{-h_o} - e^{(-vt - h_o)})$$

(2.5)

herein, $y(t)$ is the natural logarithm of the population at time t (\ln CFU/g); y_o represents the initial population number (\ln CFU/g); y_{max} is the maximum population (\ln CFU/g); h_o stands for $\mu_{max} * t_{lag}$, where t_{lag} is the lag time (hours); μ_{max} is the maximum specific growth rate (1/hours); v is the rate of increase of the critical substrate. After the inoculation, it is assumed that the critical substrate grows at the same specific rate as the cells in the exponential phase, $v = \mu_{max}$.

2.7.2 Secondary Models for the Maximum Growth Rate

Secondary models estimate the changes in the parameters of primary models with the intrinsic and extrinsic effects such as temperature, pH, and water activity (Perez-Rodriguez and Valero, 2013). These models predict the changes in the parameters of primary models such as the maximum specific growth rate and lag time.

2.7.2.1 Square-Root Models

Secondary models were suggested by Ratkowsky et al. (1982), who determined a linear relationship between the square root of the maximum growth rate and temperature.

$$\sqrt{\mu_{max}} = b * (T - T_{min}) \quad (2.6)$$

herein, b represents a regression coefficient ($^{\circ}\text{C}^{-1} \cdot \text{h}^{-1/2}$); T represents the intercept of the predicted function and the temperature axis ($^{\circ}\text{C}$); T_{min} represent the notional minimum temperature below which maximum growth rate is equal to 0 ($^{\circ}\text{C}$).

Then, this model was developed to cover the whole temperature growth range (Perez-Rodriguez and Valero, 2013).

$$\sqrt{\mu_{max}} = b * (T - T_{min})(1 - e^{c(T-T_{max})}) \quad (2.7)$$

herein, c is a parameter ($^{\circ}\text{C}$); T_{max} is the theoretical maximum temperature at which growth can be observed ($^{\circ}\text{C}$).

2.8 Quantitative Microbial Risk Assessment (QMRA)

Quantitative microbial risk assessment (QMRA) is a systematic approach to evaluate information from different sources concerning the fate of pathogens in food chain and determine the size of public health risk (Perez-Rodriguez and Valero, 2013). QMRA is as a predictive and decision-making tool and aims to determine the data gaps

in the database and requirement of additional information (Montville and Schaffner, 2005). The QMRA approach includes four components: (1) hazard identification, (2) exposure assessment, (3) hazard characterization (Dose-response assessment), and (4) risk characterization (Perez-Rodriguez and Valero, 2013). These four components are described in detail in Chapter IV.

To this date, available QMRA models for the listeriosis risk associated with fresh and fresh-cut produce throughout the supply chain are very limited. In the U.S., the preliminary QMRA framework for risk linked to ready to eat food from farm to consumption contributed with initial risk estimates for *L. monocytogenes* was conducted by USFDA (2003).

Carrasco et al. (2010) determined the risk of *L. monocytogenes* in ready-to-eat lettuce salads from farm to table in Spain. In that study, the estimated number of listeriosis cases was 10^2 and 10^5 for low and high risk subpopulations respectively. Tromp et al. (2010) assessed the risk of *E. coli* 0157:H7, *Salmonella*, and *L. monocytogenes* in ready to eat vegetables including lettuce consumed at salad bars, based on modeling supply chain logistics in the Netherlands. That study showed that the risk of listeriosis-induced fetal mortality in the perinatal population raised from $1.24 \cdot 10^{-4}$ (fixed storage time) to $1.66 \cdot 10^{-4}$. Similarly, Franz et al. (2010) assessed the risk of *E. coli* 0157:H7, *Salmonella*, and *L. monocytogenes* in leafy green vegetables consumed at salad bars in Netherlands. They estimated the average number of cases per year linked to the consumption of leafy greens at salad bars were 166, 187, and 0.3 for *E. coli* 0157:H7, *Salmonella*, and *L. monocytogenes* respectively. Puerta-Gomez et al. (2013b) assessed

risk of contamination of ready-to-eat spinach with *Salmonella* in U.S., and found that irradiation was the most effective means to reduce the number of contaminated samples from 84% to 0.1%. Ding et al. (2013) determined risk of *L. monocytogenes* on lettuce from farm to table in Korea. That study found that the final contamination levels of *L. monocytogenes* at restaurant and home were -1.50 log CFU/g and -0.146 log CFU/g respectively. They also estimated the average number of annual listeriosis cases varied from 559 to 817, depend on the different r-values employed in the exponential dose-response model. Recently, Chen et al (2013) conducted a risk assessment framework for fresh-cut cantaloupe. Although the study covered only senior population, annual estimated cases were found as 2.39.

CHAPTER III

MODELING GROWTH OF *LISTERIA INNOCUA* ON FRESH-CUT

CANTALOUPE AND FRESH-CUT ROMAINE LETTUCE

3.1 Introduction

Growth of pathogens is the most common cause of food poisoning. This is the reason why growth patterns of selected bacteria have been studied extensively. These patterns are often described with mathematical models which are valuable tools for manufacturers to develop process controls to reduce the risk of pathogen contamination in fresh and processed foods. These predictive models can also provide an estimate of the product's shelf- life based on microbial safety.

Fresh produce can be contaminated with pathogens in field or post-harvest applications. As mentioned in Chapter II, although the prevalence of *L. monocytogenes* in fresh produce is relatively lower than other common pathogens, a higher mortality rate makes this pathogen a serious problem. Furthermore, the lack of heat treatment in the process, and the ability of *L. monocytogenes* to grow at low temperatures dictate the need for researchers to understand the growth behavior and treatment response of this pathogen.

Despite its feasibility for laboratory experiments, *L. monocytogenes* cannot be safely studied in every environment because of the risk of exposure of vulnerable individuals. As a result, the use of a surrogate organism is a convenient way to understand the behavior of this pathogen in foods and food processing environments (Milillo et al. 2012). *L. innocua* is usually used as a surrogate for *L. monocytogenes* due

to their close genetic relationship (Guo et al., 2013). Recently, Omac et al. (2015) validated the use of *L. innocua* as a surrogate of *L. monocytogenes* for growth modelling in baby spinach leaves. In the current study, due to the lack of access to a biosafety level two (BSL2) lab, *L. innocua* was used as a valid surrogate of *L. monocytogenes* to obtain growth data in fresh-cut romaine lettuce and fresh-cut cantaloupe.

Therefore, the objectives of this study were to; (1) predict the growth of *L. innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe as a function of storage temperature by using different primary models, (2) compare and validate the goodness of fit of the primary models by using statistical validation tools, (3) determine the effect of washing treatments on the reduction *L. innocua* on both produces and, (4) develop and validate dynamic models for prediction of growth of *L. innocua* under different storage temperatures. These results were used as input in the quantitative risk assessment model (Chapter IV).

3.2 Materials and Methods

3.2.1 Food Material

Fresh-cut romaine lettuce (*Lactuca sativa*, var *longifolia*) was purchased from a local grocery store. All products had the same self-life date to ensure uniformity. The product was stored in the original package at 5°C for no longer than 24 hours prior to the experiments. All products were examined before experiments and the hearts showing signs of wilt and decay were discarded. 5-g of produce was weighted and dispensed into sterile stomacher bags (18 oz. Whirl Pak® bag) before inoculation. This procedure is similar to that described by Puerta-Gomez et al. (2013).

Whole cantaloupes (*Cucumis melo*), free of visual defects, were randomly purchased from a local grocery store in the summer season and stored at 5°C for less than 24 hours. Before the experiments, each cantaloupe was washed with tap water for 3 minutes and then the rind and the core were removed with a sterile knife. The fruits were subdivided into 5-g cubic shaped portions and dispensed into a sterile stomacher bag (18 oz. Whirl Pak® bag) before inoculation.

3.2.2 Initial Natural Microbiota Enumeration

Aerobic mesophilic bacteria were enumerated by spread plating on Tryptic Soy Agar (TSA) incubated at 36°C for no more than 48 hours. Yeasts and molds were quantified by spread plating on Sabouraud Dextrose Agar (pH 5.6, adjusted with 0.1% citric acid) after 5 days of incubation at 20°C (VWR International, Model 1510E, IL, USA). Plate counts of total aerobic organisms, yeasts, and molds will be evaluated only at the beginning of every experiment.

3.2.3 Inoculation and Preparation of Fresh-Cut Cantaloupe and Fresh-Cut Lettuce

For each produce, 5-g portions were distributed into sterile stomacher bags (18 oz. Whirl Pak® bag), and an initial inoculum (0.5 ml) were injected. The initial bacteria load was 10^2 CFU/ml *L. innocua* in order to mimic natural contamination (Omac et al., 2015). To dispense the inoculum uniformly, stomacher bags were shaken gently for 30 times. Different sampling times were determined for each temperature (5, 10, 25, 30, and 36°C). For each sampling time, four bags of inoculated samples were prepared. The experiment will be performed in triplicate. The samples were put in an incubator, and maintained at constant temperature (5, 10, 20, 30, and 36°C). The samples were held in

incubator for 16 days, 12 days, 60 hours, 48 hours and 36 hours at 5, 10, 20, 30, and 36 °C respectively.

3.2.4 Bacterial Cultures

Rifampicin resistant (80 µg/ml) culture of *L. innocua* (NRCC B33076) was obtained from Dr. Carmen Gomes' stock laboratory (Department of Biological and Agricultural Engineering, Texas A&M University) stored at -80°C. A loop was used to take a single inoculum from the frozen culture. Optimal temperature for incubation of *L. innocua* is around 37°C (Ryser and Marth, 2007). Inoculum was put onto Tryptose Phosphate Broth (TPB; Difco, Detroit, MI), on which the inoculum was incubated for 24 hours at 36°C. Next, the inoculum was taken with a loop and streaked on Oxford Listeria-selective agar supplemented with 80 µg/ml of rifampicin (OLR) in order to obtain single colony isolates. Inoculum was incubated at 36 °C for 24 h, and this process was repeated through two successive transfers on (OLR). Obtained colonies were rifampicin resistant. These colonies were kept on a TSA slant at 5°C, and, were used in 90 days.

3.2.5 Inoculum Preparation

The inoculum in TSA slant was taken with a loop, and transferred to TPB test tubes. The inocula in TPB tubes were incubated at 36°C for 18 hours. After 18 hours, incubated inocula were centrifuged (3000 X *g* for 15 min) and washed with Difco buffered peptone water for three times consecutively. Afterwards, each pellet was suspended in 0.1% peptone water (PW). To determine the initial concentration, the OD₆₀₀ of the cell suspensions was adjusted to 0.5 of absorbance for bacterial preparation.

Serial dilutions of the suspension were made in test tubes of 9 ml PW, in order to verify initial concentration will be 10^7 CFU/ml. Subsequently, the suspension was plated on OLR, and incubated at 36°C until countable visible black colonies obtained. To acquire 10^2 CFU/ml of *L. innocua* and strains, series of dilutions of initial population in PW were prepared.

3.2.6 Washing and Sanitizing Treatments

In this study, romaine lettuce was washed after cutting, and cantaloupe was washed as whole to simulate standard industrial practices. Four bags of romaine lettuce samples (5-g per 18 oz Whirl Pak® bag) with different initial inoculum loads (10^3 , 10^4 , and 10^5 CFU/ml) of *L. innocua* were washed with tap water for 10-minutes at room temperature. During the treatment, the washing solution was sometimes stirred to increase the water contact. Then, four different bags of samples (5-g per sample in 18 oz Whirl Pak® bag) with same initial loads as for the water washing treatment were treated with 200 mg/liter of chlorinated water at pH 7.0 (reduced with 0.1 N of HCl) for 10-minutes at room temperature. Similarly, the solution was stirred occasionally during the treatment. After the treatment, each 5-g sample was placed in an 18-oz. stomacher bag and kept at 5°C for 2 hours (Omac et al. 2015). Then, the number of microorganisms remaining on the surface of the products was determined by using microbial enumeration methods.

Washing of cantaloupe was carried out as described by Vadlamudi et al. (2012). 500 ml bacterial solutions were prepared as described above and transferred into a bowl containing 4500 ml % 0.1 peptone water solution to produce 5 liters of solution. Whole

cantaloupes were submerged into the solution for 3 minutes, and gently agitated with gloves. After every sample, gloves were changed to eliminate the risk of cross contamination. The fruit samples were allowed to dry at room temperature in a biosafety cabinet (Labconco Purifier Logic Class II Type 2, Kansas City, MO) for 2 hours prior to the washing treatments. Sanitizers were prepared as described before. All samples (including those subject to chlorine washing) were washed for 3 minutes under tap water. After that, chlorine washing samples were submerged into 5 liters of a 200 mg/liter chlorine solution, and rotated for 5 minutes. Next, five samples were collected with a sterile cork borer (1 cm² area), and a sterile scalpel and placed inside an 18 oz Whirl Pak® bag (Nasco, Fort Atkinson, WI) containing 99 ml 0.1 % of PW. Series of dilutions were prepared to enumerate the *L. innocua* cells. The colonies were divided by 5 to determine the count in CFU/cm².

3.2.7 Microbial Enumeration

Microbial enumeration was conducted as described by Omac et al. (20015). Each 5-g sample of fresh produce inoculated with *L. innocua* was hand pummeled with 45 ml of Difco buffered peptone water (BPW; Difco, Detroit, MI) in an 18 oz Whirl Pak® bag until samples were reduced to small pieces, allowing the internal structure to be exposed. Samples of 1 ml from the original Whirl Pak® bag and 0.1 ml from serial dilution in 0.1 % of PW were plated in duplicate (0.1 ml) on Oxford *Listeria*-selective agar supplemented with 80 µg/ml of rifampicin for *Listeria innocua* enumeration. Plates were incubated for 24 h at 37°C. After incubation, visible colonies were enumerated with the use of a magnifier counter (detection limit was 10 CFU/g of sample).

3.2.8 Isothermal Growth Data

In order to assess bio-kinetic growth temperatures at different levels of initial *L. innocua* inoculum and natural microbiota, growth data of *Listeria innocua* were determined at five different temperatures (5, 10, 20, 30 and 36°C) were Four samples (18 oz. stomacher bags) were prepared for each sampling time. Each of the inoculated samples was placed in an incubator (VWR International, Model 1510E, IL, USA), and maintained at constant temperature. After waiting 10 min for temperature stabilization, all samples were taken from the stomacher bags to calculate the initial inoculum using microbial enumeration techniques. Three independent replications were carried out at each temperature.

3.2.9 Microbial Growth Models

3.2.9.1 Primary Model

In this study, Baranyi-Roberts (Eq. 2.4), Gompertz (Eq. 2.2), and Logistic (Eq. 2.1) models were evaluated to determine their feasibility to describe the four main growth phases, i.e. lag, acceleration, deceleration, and stationary. DMFit Excel Add-In software (Norwich, UK) and SigmaPlot software (Systat Software Inc. San Jose, CA) were be used to fit the models (Chapter II, Section 2.7.1)

Statistical analysis were run to validate the acceptability of the models. Hence, the ability of the model to accurately predict the growth of *L. innocua* in both produces as a function of temperature was evaluated by the root mean square error (RMSE) and coefficient of determination (R^2). RMSE value is the difference between observed and

predicted data and values close to zero are desirable as they indicate that predicted values are very close to the observed values. R^2 was calculated as follows,

$$R^2 = 1 - \frac{SSR}{SST} \quad (3.1)$$

Where, SSR is the sum of squares of residuals and SST is the total sum of squares. The root mean squared error ($RMSE$) was calculated as,

$$RMSE = \sqrt{\frac{SSE}{N-p}} \quad (3.2)$$

Herein, N is the number of observations, and p is the number of model parameters. The above statistical quantities were calculated at each storage temperature after fitting the growth data into Eqs. (2.1), Eq. (2.2), and Eq. (2.4) (Chapter II, section 2.7.1).

3.2.9.2 Secondary (Dynamic) Models

The modified Ratkowsky equation (Eq. 2.6, Chapter II) was used to describe the effect of temperature on the maximum growth rate, μ_{max} (log CFU/g/h), by,

$$\sqrt{\mu_{max}} = b * (T - T_{min}) \quad (2.6)$$

Herein, the parameter T_{min} represents the theoretical minimum temperature at which the target bacterium can grow, and the parameter b is a regression coefficient. Equation (2.6) represents the microbial growth rate up to the optimum growth temperature for any temperature change (e.g., a dynamic model).

To predict lag time, t_{lag} , an inverse Ratkowsky-type model was used, (from Eq. 2.7, Chapter II),

$$t_{lag} = (c * (T - T_{\min}))^{-2} \quad (3.3)$$

Where c is a regression coefficient.

3.2.9.3 Validation of Dynamic Models

Three validation tests were performed: (1) Bias Factor (B_f), (2) Accuracy Factor (A_f), and (3) the standard error of prediction expressed as a percentage (%SEP).

The bias factor is a measurement of the model prediction bias that computes the differences between the means of actual and predicted values (Ding et al., 2010),

$$B_f = 10^{(\sum_{i=1}^n \log(\frac{O}{P})/n)} \quad (3.6)$$

The accuracy factor measures the accuracy of calculation as the proximity of predicted values to the actual values (Ding et al., 2010),

$$A_f = 10^{(\sum_{i=1}^n |\log(\frac{P}{O})|/n)} \quad (3.7)$$

The Standard Error of prediction is a relatively typical deviation of the mean prediction values,

$$\%SEP = \frac{100}{Average(O)} \sqrt{\frac{\sum(O-P)^2}{n}} \quad (3.8)$$

where, O is the observed value; P is the predicted value; and n is the number of observations and predictions.

3.2.10 Statistical Analysis

Data analysis for comparison between the products and washing treatments was performed using SPSS software (version 20.0 for Windows, 2011). Statistical differences between variables were analyzed for significance by one-way ANOVA using

Tukey's multiple range tests. In addition, growth models for both produce were compared by independent sample t-test. Statistical significance was determined at the $P < 0.05$ level.

3.3 Results and Discussion

3.3.1 Effect of Washing Treatments on Reduction of *L. innocua* Population

Since cantaloupes were washed as whole to simulate industrial practices, the log reduction in *L. innocua* population on the surface of the cantaloupe was determined as log CFU/cm². As expected, chlorine washing was significantly more effective ($p < 0.05$) than water washing for both products (Table 3.1). Furthermore, the level of initial population load had no effect ($p > 0.05$) on the effectiveness of the chlorine washing treatment of romaine lettuce. Due to uncertainty of attachment of *L. innocua* to cantaloupe surface, the effect of initial population level could not be tested. The reduction of *L. innocua* population on cantaloupe rinds was significantly lower ($p < 0.05$) than on fresh-cut romaine lettuce, probably due to the different contact surfaces. For instance, Annous et al (2005) reported that the netted surface of cantaloupe provides pathogens greater attachment surfaces thus reducing the effectiveness of sanitizers.

Table 3.1. Effect of washing treatment on the log-reductions of *L. innocua* inoculated on fresh-cut romaine lettuce and whole cantaloupe at room temperature.

Produce	Initial population load (log CFU/g) ¹	Population Reduction (log CFU/g) ¹	
		Water only	Chlorinated water
<i>Fresh-cut romaine lettuce</i>	3	0.63 ^a ² (0.17)	0.89 ^b (0.04)
	4	0.55 ^a (0.16)	1.05 ^b (0.11)
	5	0.53 ^a (0.05)	1.01 ^b (0.12)
<i>Cantaloupe surface</i>	4.4 (0.67)	0.18 ^c (0.15)	0.57 ^d (0.19)

¹: Reduction and initial population load in cantaloupe surface is determined as log CFU/cm²

²: means are values of 8 replication; Standard deviation

^{a,b,c}: Means within a column or row, which are not followed by a common superscript letter are significantly different ($P < 0.05$).

A considerable amount of literature has been published on the effect of washing treatments on fresh-cut vegetables. In the case of leafy greens such as lettuce, for example, Beuchat et al. (2004) inoculated different amounts of *L. monocytogenes* onto romaine lettuce, then compared water only and chlorinated water treatments. The authors observed reductions of 0.38 and 1.05 logs in populations treated with water and chlorine, respectively. Another study found that washing with chlorine (200-ppm, 10 min) reduced *L. monocytogenes* on shredded lettuce by 1.7 CFU/g (Zhang and Farber, 1996). Likewise, Burnett et al. (2004) determined the reduction of *L. monocytogenes* population on cut iceberg to be 0.6 and 1.76 log CFU per lettuce piece for water and 200 ppm

chlorine, respectively. Omac et al. (2015) inoculated different loads of *L. monocytogenes* onto fresh baby spinach leaves and confirmed that washing with chlorine was more effective with 0.97, 1.05 and 0.87 log CFU/g reductions for 10^3 , 10^4 , and 10^5 log CFU/g population initial loads, respectively. All these findings corroborate the suggestion of Matthews (2009) that chemical sanitizers generally reduce the bacteria population between 1 and 2 logs in fresh vegetables.

The number of studies on the effect of sanitizers on the population of *L. monocytogenes* in cantaloupe are limited compared to fresh-cut lettuce. The lack of studies on this pathogen can be explained by the fact that *L. monocytogenes* is not as common on cantaloupe as other pathogens such as *Salmonella*. However, due to recent outbreaks, an increase in treatment studies should be expected. Ukuku and Fett (2002) inoculated *L. monocytogenes* onto cantaloupe rinds and then treated the samples with 1000 ppm chlorine resulting in more than 2 log CFU/g reduction. Although our results differ from that study, this inconsistency may be due to the difference in free chlorine level. In current study, free chlorine level was 5 time less than Ukuku and Fett (2002).

3.3.2 Growth Models of *L. innocua*

3.3.2.1 Fresh-Cut Romaine Lettuce

All the three models (Baranyi-Roberts (Eq. 2.4), Gompertz (Eq. 2.2) and Logistic (Eq. 2.1) yielded RMSE and R^2 values that indicate their goodness of fit (Tables 3.2 to 3.4). The Logistic model yielded 0.993 R^2 value while the other models provided 0.98 R^2 . In case of RMSE, the Baranyi-Roberts model provided the lowest RMSE value, but the difference between the models was not significant ($p > 0.05$). Overall, it can be said

that all models fitted the data well for both products at all temperatures (Figures A.11 to A.19, Appendix A).

Table 3.2 Estimated maximum population density, maximum growth rate, and lag time of *L. innocua* inoculated on fresh-cut romaine lettuce by using Baranyi-Roberts model (Eq. 2.4).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.34 ±0.005 ^g	4.96 ±0.007	0.011 ±0.0005	57.86	0.983	0.1
10	2.36 ±0.06	5.65 ±0.3	0.02 ±0.001	0	0.975	0.16
25	2.02 ±0.03	6.71 ±0.07	0.265 ±0.03	0	0.962	0.3
30	2.81 ±0.09	6.97 ±0.11	0.314 ±0.03	0	0.971	0.23
36	2.73 ±0.52	7.73 ±0.31	0.314 ±0.043	0	0.989	0.163

^by₀: Initial population density (log CFU/g)

^cy_{max}: Maximum population density (log CFU/g)

^dμ_{max}: Maximum growth rate (log CFU/g/h)

^et_{lag}: Lag time (hours)

^fRMSE: Root mean square root (Eq. 3.2) (log CFU/g)

At 5°C, the three models yielded similar (p>0.05) values of the maximum population density (y_{max}) and maximum growth rate (μ_{max}) (Tables 3.2-3.5). At 10°C

however, each model provided a different ($p < 0.05$) maximum growth rate (μ_{\max}). Also, the difference in maximum population density (y_{\max}) estimated by Gompertz (Eq. 2.2) and Logistic (Eq. 2.1) models was significant ($p < 0.05$). Perni et al. (2005) reported that when *L. monocytogenes* was grown in liquid media, Gompertz and Logistic models provided significantly different growth rates than Baranyi and Roberts model. Authors suggested that since Gompertz and Logistic models were empirical models, they might be affected by the absence of lag time. At 25°C, the μ_{\max} calculated with the Baranyi and Roberts model was significantly lower than other models ($p < 0.05$) whereas there was no difference among the models in terms of y_{\max} ($p > 0.05$). At 30°C, the Logistic model estimated significantly higher y_{\max} values ($p < 0.05$). The maximum growth rates (μ_{\max}) provided by Baranyi-Roberts and Logistic models were also different ($p < 0.05$) at this temperature. Lastly, there was no difference among the three models at 36°C. Lag time was not observed above 5°C, suggested that *L. innocua* can begin exponential growth after a very short lag or no lag time. Although the Baranyi and Roberts model yielded significantly lower lag time than other models ($p < 0.05$), when compared with the raw data, this estimation was more realistic compared to the other models.

As expected, both maximum growth rate and maximum population density increased significantly ($p < 0.05$) with an increase in temperature. The effect of temperature was more drastic at 10°C and above. This result is explained by the fact that *L. innocua* is mesophilic, and grows much faster when the temperature approaches the optimum conditions which is 37°C (Lasagabaster and de Maranon, 2014).

Table 3.3. Estimated maximum population density, maximum growth rate, and lag time of *L. innocua* inoculated on fresh-cut romaine lettuce by using Gompertz Model (Eq. 2.2).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.398 ±0.025 ^g	4.85 ±0.2	0.014 ±0.0015	150.53	0.995	0.065
10	2.01 ±0.1	5.83 ±0.3	0.023 ±0.0005	0	0.991	0.71
25	2.22 ±0.43	7.035 ±0.23	0.314 ±0.0022	0	0.991	0.14
30	2.74 ±0.067	7.11 ±0.12	0.4 ±0.05	0	0.99	0.15
36	2.58 ±0.38	7.85 ±0.33	0.53 ±0.14	0	0.998	0.68

^by₀: Initial population density (log CFU/g)

^cy_{max}: Maximum population density (log CFU/g)

^dμ_{max}: Maximum growth rate (log CFU/g/h)

^et_{lag}: Lag time (hours)

^fRMSE: Root mean square root (Eq. 3.2) (log CFU/g)

Overall, these results match with previous studies. For instance, Koseki and Isobe (2005a) and Carrasco et al (2008) determined similar y_{max} values for *L. monocytogenes* on iceberg lettuce for temperatures between 5 and 25°C. Lu et al. (2007)

found bacterial growth rate on fresh-cut lettuce 0.0176, 0.043, and 0.0613 log CFU/g/h at 0, 4, and 25°C respectively.

Table 3.4. Estimated maximum population density, maximum growth rate, and lag time of *Listeria innocua* inoculated on fresh-cut romaine lettuce by using Logistic Model (Eq. 2.1).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.40 ±0.004 ^g	4.97 ±0.08	0.013 ±0.0013	158.66	0.996	0.06
10	2.41 ±0.01	5.99 ±0.33	0.025 ±0.0002	0	0.993	0.72
25	2.05 ±0.01	7.03 ±0.09	0.33 ±0.023	0	0.994	0.14
30	2.98 ±0.01	8.3 ±0.04	0.45 ±0.04	0	0.996	0.1
36	2.60 ±0.35	7.88 ±0.31	0.43 ±0.05	0	0.999	0.68

^by₀: Initial population density) (log CFU/g)

^cy_{max}: Maximum population density (log CFU/g)

^dμ_{max}: Maximum growth rate (log CFU/g/h)

^et_{lag}: Lag time (hours)

^fRMSE: Root mean square root (Eq. 3.2) (log CFU/g)

Fang et al. (2013) reported close growth rates at 25, 30, and 35°C for *L. monocytogenes* on RTE lettuce. Wang et al. (2013) stated that μ_{\max} values for *L. monocytogenes* growth on cabbage were between 0.008 and 0.320 log CFU/g/h at 4-30°C. Omac et al. (2015) reported that there is no difference between the growth of *L. innocua* and *L. monocytogenes* in fresh baby spinach. Although same experiments should be conducted with *L. monocytogenes*, these findings are encouraging and supports the idea that *L. innocua* can be used as a surrogate for *L. monocytogenes* in modeling studies of pathogen growth in fresh produce.

3.3.2.2 Fresh-Cut Cantaloupe

Like romaine lettuce, there was no difference in maximum growth rate (μ_{\max}) and maximum population density (y_{\max}) values at 5°C for *L. innocua* in fresh cut-cantaloupe (Tables 3.5 to 3.7).

Similar to romaine lettuce, at 10°C, Baranyi and Roberts model provided significantly lower ($p < 0.05$) maximum population density (y_{\max}) than the other models ($p < 0.05$). This difference can be associated with the absence of lag stage. As *L. innocua* immediately began to grow exponentially at 10°C, empirical models might overestimated the parameters.

Table 3.5. Estimated maximum population density, maximum growth rate, and lag time of *L. innocua* inoculated on fresh-cut cantaloupe by using Baranyi and Roberts model (Eq. 2.4).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.39 ±0.06 ^g	5.30 ±0.1	0.017 ±0.005	80.73	0.997	0.042
10	2.36 ±0.1	7.45 ±0.09	0.037 ±0.04	0	0.989	0.18
25	2.60 ±0.09	7.17 ±0.06	0.27 ±0.02	0	0.976	0.23
30	3.04 ±0.08	8.09 ±0.038	0.31 ±0.03	0	0.974	0.26
36	2.81 ±0.02	8.34 ±0.087	0.37 ±0.018	0	0.99	0.18

^by₀: Initial population density) (log CFU/g)

^cy_{max}: Maximum population density) (log CFU/g)

^dμ_{max}: Maximum growth rate log CFU/g/h)

^et_{lag}: Lag time) (hours)

^fRMSE: Root mean square root (Eq. 3.2) (log CFU/g)

Similarly, at 25°C, maximum population densities (y_{max}) estimated by Baranyi-Roberts and Logistic models were different (p<0.05), whereas there was no difference in maximum growth rate (μ_{max}). Finally, there was no difference between the model

parameters at 36°C. Like fresh-cut romaine lettuce, lag time was observed only at 5°C. This result emphasize the fact that maintaining the cold chain is crucial in the fresh-cut cantaloupe processing and storage, as the pathogen grows immediately in higher temperatures. Fang et al. (2013) also reported that when the growth of *L. monocytogenes* was modeled on fresh-cut cantaloupe, the lag time was not observed at 8°C and above.

Table 3.6. Estimated maximum population density, maximum growth rate, and lag time of *Listeria innocua* inoculated on fresh-cut cantaloupe by using Logistic Model (Eq. 2.4).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.46 ±0.04 [§]	5.37 ±0.12	0.02 ±0.04	91.37	0.996	0.07
10	2.51 ±0.14	8.06 ±0.09	0.051 ±0.007	0	0.99	0.25
25	2.75 ±0.03	7.42 ±0.1	0.32 ±0.026	0	0.995	0.11
30	2.98 ±0.01	8.30 ±0.03	0.45 ±0.04	0	0.994	0.14
36	3.05 ±0.01	8.53 ±0.09	0.47 ±0.03	0	0.989	0.21

^by₀: Initial population density (log CFU/g)

^cy_{max}: Maximum population density (log CFU/g)

^dμ_{max}: Maximum growth rate (log CFU/g/h)

^et_{lag}: Lag time (hours)

^fRMSE: Root mean square root (log CFU/g)

Results from this study show consistency with previous studies, in which lag time was not observed above 5°C (Fang et al., 2013; Danyluk et al., 2014). Also, Li et al. (2013) showed that unlike *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 did not grow on fresh-cut cantaloupe at low temperatures. Although *Salmonella* grew much faster than *L. monocytogenes* at 20°C and above, our results were expectedly higher at lower temperatures. This study confirms that *Listeria spp.* is a bigger problem in fresh produce at lower temperatures. When differences in the model parameters as a function temperature were examined, it was seen that maximum growth rate (μ_{\max}) and maximum population density (y_{\max}) values increased significantly as the temperature increased with some exceptions. There was no difference in maximum growth rate (μ_{\max}) values at 30°C and 36°C. Although maximum population density (y_{\max}) was increased with time, this increase was not significant, and values were mostly stable at 10°C and above. These results are consistent with similar studies. Fang et al. (2013) reported that on fresh cut cantaloupe maximum population density was of *L. monocytogenes* was similar between 10°C and 40°C. In that study, maximum population density was calculated 8 ± 0.5 log CFU/g which is similar to the value used in this study.

To assess the differences in product-based model parameters, an independent sample *t* test was conducted at each temperature. Unsurprisingly, at every temperature, the maximum population density of *L. innocua* in fresh-cut cantaloupe was higher ($p < 0.05$) than in fresh-cut romaine lettuce. Similarly, maximum growth rate (μ_{\max}) of *L. innocua* was higher ($p < 0.05$) in fresh-cut cantaloupe compared to romaine lettuce except

at 25°C and 30°C. At these temperatures, while there was a difference between the growth rates, that difference was not significant ($p>0.05$).

Table 3.7. Estimated maximum population density, maximum growth rate, and lag time of *L. innocua* inoculated on fresh-cut cantaloupe by using Gompertz Model (Eq. 2.2).

Temperature (°C)	^b y ₀ (log CFU/g)	^c y _{max} (log CFU/g)	^d μ _{max} (log CFU/g/hr)	^e t _{lag} (Hours)	R ²	^f RMSE (log CFU/g)
5	2.46 ±0.026 ^g	5.36 ±0.13	0.02 ±0.004	86.84	0.996	0.07
10	2.45 ±0.13	7.98 ±0.09	0.047 ±0.007	0	0.991	0.19
25	2.71 ±0.06	7.30 ±0.12	0.3 ±0.03	0	0.99	0.16
30	2.93 ±0.014	8.27 ±0.025	0.47 ±0.13	0	0.992	0.17
36	2.80 ±0.04	8.53 ±0.053	0.45 ±0.03	0	0.99	0.2

^by₀: Initial population density) (log CFU/g)

^cy_{max}: Maximum population density (log CFU/g)

^dμ_{max}: Maximum growth rate (log CFU/g/h)

^et_{lag}: Lag time (hours)

^fRMSE: Root mean square root (Eq. 3.2) (log CFU/g)

Because *L. innocua* is a mesophilic bacterium, it is possible that at the optimum temperature range the differences in growth media do not play a significant role.

Although the differences in model parameters related to the food product can be related to many variables, the most likely reasons are the differences in pH and sugar content (Hoelzer 2012b).

3.3.3 Secondary (Dynamic) Growth Models

3.3.3.1 Fresh-Cut Romaine Lettuce

The effect of temperature on maximum growth rate (μ_{\max}) described by Ratkowsky model (Eq. 2.13) is shown in Figure 3.1. Baranyi and Roberts model was modified to find a lag time at higher temperatures (Table 3.8). In addition, the coefficients of Eq. (3.4) and Eq. (3.5) are presented in Tables 3.9 and 3.10, respectively.

Ding et al. (2010) reported the b coefficient of the Ratkowsky model as 0.014 for Gompertz model. Sant'ana et al. (2012) determined the coefficient as 0.0144 for *L. monocytogenes* in ready to eat lettuce. In another study, the coefficient b was found to be 0.016 after growth of *L. monocytogenes* was modeled with Baranyi and Roberts model in lettuce (Koseki et al., 2005). Among the three primary growth models, the Baranyi and Roberts model provided the value closest that that found in the literature ($b= 0.015$, Table, 3.8). The reason for differences among the predictive growth models is discussed in Section 3.4.5.

Table 3.8. The b coefficient of Eq. (2.13)^a used to predict the values of maximum growth rate as a function of temperature *L. innocua* inoculated in fresh-cut romaine lettuce.

Primary Growth Model	^b b (log CFU/g/hr/°C)	^c T_{min} (°C)	R ²
Baranyi and Roberts	0.0152	-4.26	0.93
Gompertz	0.0179	-4.26	0.954
Logistic	0.0177	-4.26	0.93

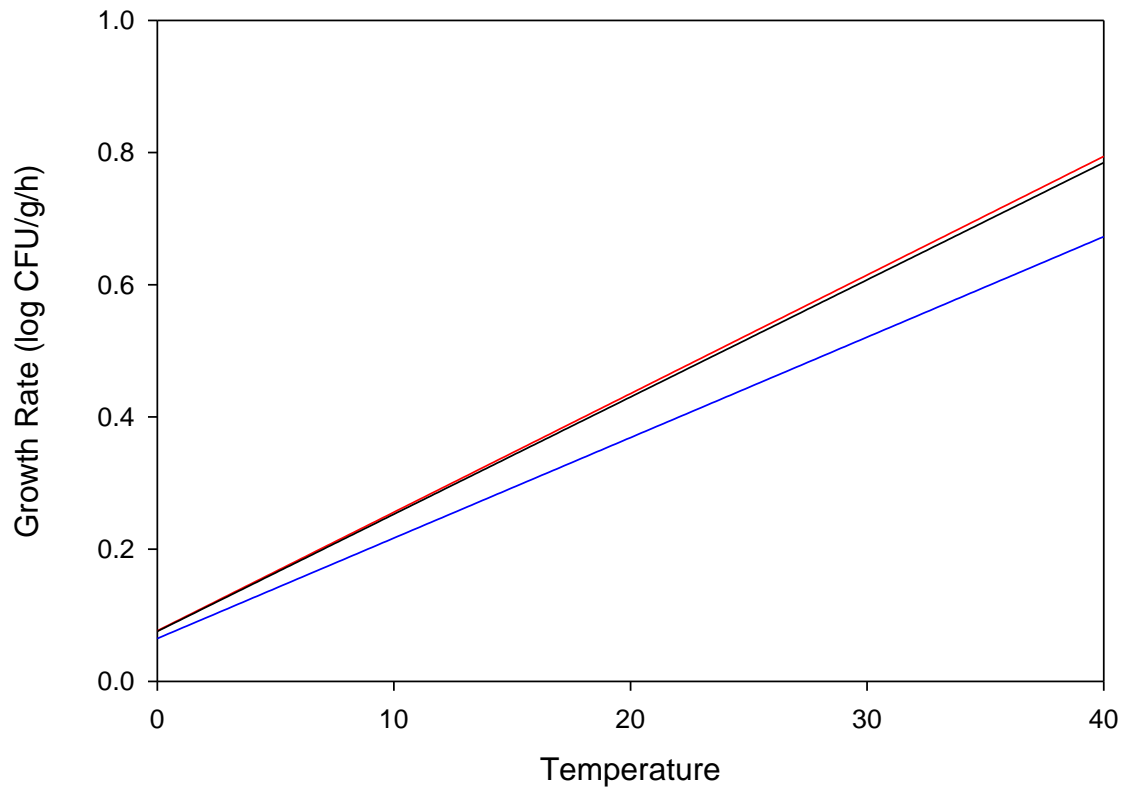
$$^a\sqrt{\mu_{max}} = b * (T - T_{min})$$

^b b : coefficient of Eq. (2.6)

^c T_{min} : Minimum growth temperature of *L. monocytogenes* (°C) (Koseki and Isobe, 2005a)

The goodness of fit of the secondary models was evaluated with R² values. R² values of the μ_{max} were 0.93, 0.95, and 0.93 for Baranyi and Roberts (Eq. 2.4), Gompertz (Eq. 2.2), and Logistic (Eq. 2.1) models respectively. These results indicated that all primary models successfully described the pathogen growth. However, as temperature increased, the difference between the growth rates provided by Baranyi and Roberts model and others are also increased (Figure 3.1), which suggested that R² values alone might not be enough to determine the goodness of fit, and further validation tools are needed.

Figure 3.1. Effect of temperature on the maximum growth rate (μ_{\max}) of *L. innocua* in fresh-cut romaine lettuce (Eq. 2.13)

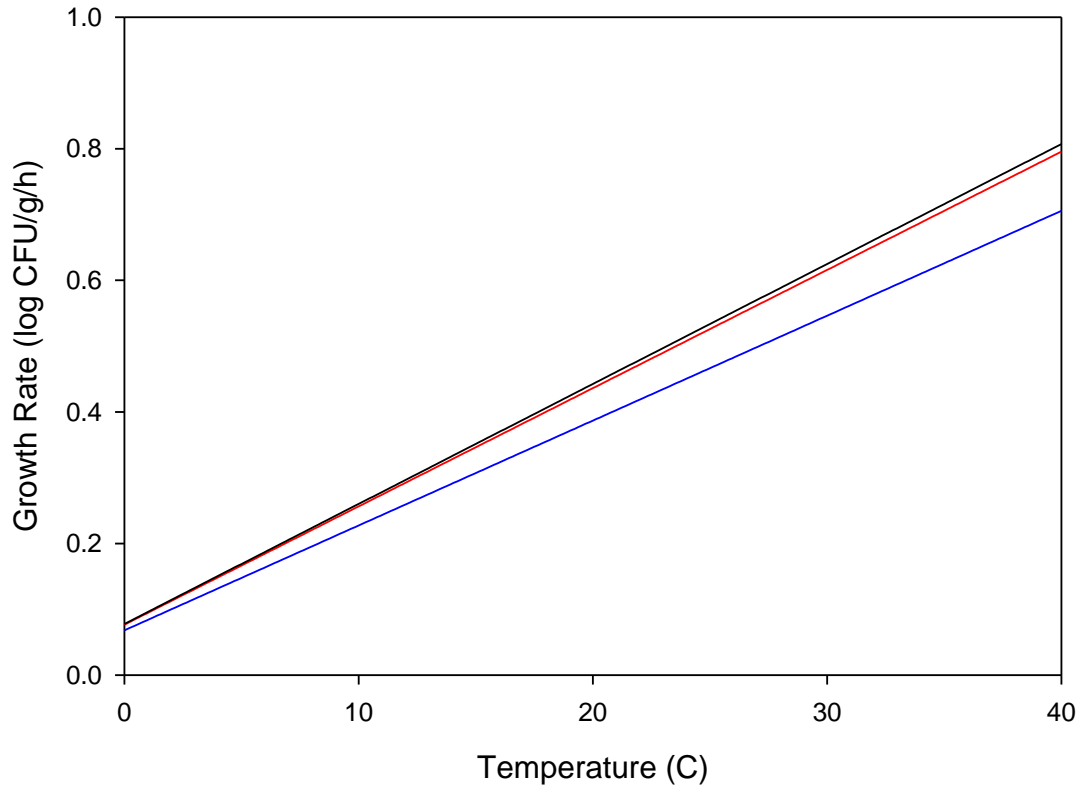


Blue: Baranyi Model (2.4)
Black: Logistic Model (2.1)
Red: Gompertz Model (2.2)

3.3.3.2 Fresh-Cut Cantaloupe

Like in the case of fresh-cut romaine lettuce, the Ratkowsky model (Eq. 2.13) was used for secondary modeling of all primary growth models of the pathogen in fresh-cut cantaloupe (Table 3.9). The effect of temperature on maximum growth rate (μ_{\max}) was shown in (Figure 3.2).

Figure 3.2. Maximum growth rate (μ_{\max}) of *L. innocua* on fresh-cut cantaloupe as a function of temperature (Eq. 2.13)



Blue: Baranyi Model (Eq. 2.4)
Black: Logistic Model (Eq. 2.1)
Red: Gompertz Model (Eq. 2.2)

R^2 values of the μ_{\max} were 0.97, 0.95, and 0.97 for Baranyi and Roberts, Gompertz, and Logistic models respectively. Although R^2 values were slightly lower than fresh-cut romaine lettuce, the models were in the 'good fit' range. However, the gap between Baranyi and Roberts, and the other models increased with the temperature. This pattern was similar to fresh-cut romaine lettuce model.

Table 3.9. The b coefficient of Eq. (2.6)^a used to predict the values of maximum growth rate as a function of temperature *L. innocua* inoculated in fresh-cut cantaloupe.

Model	^b b (log CFU/g/hr/°C)	^c T_{min} (°C)	R^2
Baranyi-Roberts	0.0159	-4.26	0.972
Gompertz	0.018	-4.26	0.959
Logistic	0.0182	-4.26	0.97

^a $\sqrt{\mu_{max}} = b * (T - T_{min})$

^b b : The coefficient of Eq. (2.6)

^c T_{min} : Minimum growth temperature of *L. monocytogenes* (°C) (Koseki and Isobe, 2005a)

It can be said that regardless of growth media, Gompertz and Logistic models overestimated the parameters at high temperatures. Furthermore, for Baranyi and Roberts model, R^2 values of the t_{lag} model were evaluated for both products, and found 0.95 and 0.92 for cantaloupe and lettuce respectively. With the high R^2 values it can be argued that the Ratkowsky model successfully describes the relationship between the maximum growth rate (μ_{max}) and temperature. This model also provides valuable information on pathogen growth under fluctuating temperatures by estimating specific growth rates.

Table 3.10. Coefficients of ^aEq. (2.7) used to predict the values of lag time as a function of temperature for *L. innocua*

Product	^b c (1/hours*°C)	^c T _{min} (°C)	R ²
Cantaloupe	0.115	-4.26	0.953
Romaine Lettuce	0.11	-4.26	0.921

^a: $t_{lag} = (c * (T - T_{min}))^{-2}$

^bc : Coefficients of ^aEq. (2.7)

^cT_{min} : Minimum growth temperature of *L. monocytogenes* (°C) (Koseki and Isobe, 2005a)

Danyluk et al. (2014) determined the coefficient (b) for *L. monocytogenes* in fresh-cut cantaloupe as 0.0186. The authors used a Baranyi and Roberts model to predict the growth curves. Although coefficient (b) of Ratkowsky model is slightly higher than fresh-cut romaine lettuce, this small difference has a significant impact in specific growth rates. Moreover, compared to chemical properties, higher sugar content and lower acidity makes cantaloupe a more suitable host to *L. innocua* than fresh-cut romaine lettuce (Hoelzer et al., 2012). A similar difference was observed in the lag time (t_{lag}) modelling coefficients. Although the difference between the coefficients is 0.005 (1/h/°C), significant differences were observed when lag time (t_{lag}) was estimated in

different temperatures. The differences between the growth rate (μ_{max}) and lag time (t_{lag}) coefficients were also discussed in the next chapter.

All in all, modelling of *L. innocua* provided similar results with *L. monocytogenes* reported in literature. Also, the current study determined that *L. innocua* shows a very little or no lag time at 10°C and above temperatures. Danyluk et al. (2014) also reported that when the growth of *L. monocytogenes* was modeled in fresh-cut cantaloupe, no lag time was observed.

3.3.4 Model Validation

To evaluate the overall performances of the dynamic models, the maximum growth rate (μ_{max}) values of *L. innocua* for both products obtained from the primary models were compared with the predictions obtained with the secondary models.

For fresh-cut romaine lettuce, the bias factor (B_f) values were 1.00, 1.10, and 0.98 in the same order (Table 3.11). Ross et al. (1996) and Valero et al. (2007) recommended that the bias factor (B_f) should range from 0.90 to 1.05 to be considered as good for determining the growth parameters. Similarly, Ding et al. (2010) proposed that B_f values in range of 0.70 to 1.15 were considered as ‘acceptable’. The results of this study indicated that the Baranyi and Roberts, and Logistic models provided good fit of growth data while the Gompertz model was only in the ‘acceptable’ range.

Table 3.11. Validation indices of developed models for maximum growth rate of *L. innocua* in fresh-cut romaine lettuce.

Growth Model	B_f (Eq. 3.6)	A_f (Eq. 3.7)	%SEP (Eq. 3.8)
Baranyi and Roberts	1	0.96	10.2
Gompertz	1.1	1.05	8.84
Logistic	0.98	1.01	8.86

Standard error of prediction is the percentage of the difference between the dynamic model and primary model. %SEP values were 10.2, 8.8, and 8.8 for Baranyi and Roberts, Gompertz, and Logistic models, respectively. Although all growth models could be fit the experimental data very well, the Logistic model gave the best results. This result was rather unexpected. A possible explanation is that although at high temperatures Logistic model overestimated the parameters, the model gave the closest fit to experimental data at 5°C. The accuracy factor (A_f) values were 1.01, 1.05, and 1.03 in the same order. A_f values should be close to 1.0 to provide good fit of the data (Ding et al., 2010). Hence, the A_f values for all tested models were in good fit range for both products. Omac et al. (2015) found the A_f values of Baranyi and Roberts model as 1.43

and 1.37 for *L. monocytogenes* and *L. innocua* respectively. These results indicated that model performance might be affected from growth media as well. Ding et al. (2010) found A_f value 1.1 for Gompertz model of *L. monocytogenes* growth in iceberg lettuce. According to accuracy factor Baranyi and Roberts model provided the overall best fit.

Table 3.12 Validation indices of developed models for maximum growth rate of *L. innocua* in fresh-cut cantaloupe.

Model	B_f (Eq. 3.6)	A_f (Eq. 3.7)	%SEP (Eq. 3.8)
Baranyi and Roberts	0.96	1.03	9.6
Gompertz	1.09	1.15	10.1
Logistic	0.95	1.04	8.9

For fresh-cut cantaloupe, the bias factor (B_f) and the standard error of prediction (%SEP) of the predictive models were 0.96, 1.09, and 0.95 and 9.6, 10.1, and 8.9 for Baranyi-Roberts, Gompertz, and Logistic, respectively (Table 3.12). Secondly, the accuracy of the models (A_f) in fresh-cut cantaloupe were 1.03, 1.15, and 1.04, respectively. Like in the case of fresh-cut romaine lettuce, the Baranyi and Roberts model provided the best bias and accuracy factor values, while the %SEP value of

Logistic model was the best among the models. These results suggested that %SEP value might be affected by the model performance at specific temperature points and might not be a good validation tool alone.

As mentioned Section 3.4.4.1., Baranyi and Roberts model yielded results similar to previous studies (Koseki and Isobe, 2005; Ding et al., 2010; Sant'ana et al., 2012). Moreover, the 'b' coefficient of the Ratkowsky model obtained from the Baranyi and Roberts model was the lowest, suggesting a more realistic growth estimation. Although all three models provided good fit overall, the bias and accuracy factors indicate that the Baranyi and Roberts model predicts values closest to the actual data. At high temperatures (10 to 36°C), both Gompertz and Logistic models overestimated the growth rate and maximum population density, probably because of the lack of adequate stationary phase at higher temperatures. This confirms the report by Buchanan et al. (1997) who observed that, compared to Baranyi and Roberts model, Gompertz model tends to overestimate the maximum population density when the stationary phase has limited data points. Perez-Rodriguez and Valero (2013) also reported that empirical models like Gompertz and Logistic models, overestimate the growth rate systematically. Hence, the findings from this study are consistent with the previous studies on the overestimation issue with Gompertz and Logistic models. Especially for the specific growth rates obtained from dynamic models, dramatic differences may occur between the models. Based on this analysis, the Baranyi-Roberts model was chosen to estimate the growth of the pathogen for use in the Quantitative Risk Assessment model (Chapter IV).

3.4 Conclusions

Washing treatments reduced the *L. innocua* load in fresh-cut romaine lettuce around 1 log CFU/g. On the other hand, the treatments caused around 0.50 less log reduction in cantaloupe, probably due to the different surface characteristics of the produces. Chlorine washing was more than 300% effective than washing with water only in reducing microbial load on fresh-cut cantaloupe, whereas chlorinated water resulted in 71% more log reductions than water only washing in fresh-cut romaine lettuce.

As expected, *L. innocua* grew faster as the incubation temperature increased. The highest increase in maximum growth rate (μ_{\max}) was observed between 10 and 25°C. The lag time was not observed at 10°C and above, suggesting *L. innocua* can start exponential growth immediately on fresh-cut produce.

The three models evaluated in this study provided a good fit of the growth data. Moreover, all models provided similar growth rate (μ_{\max}) and maximum population density (y_{\max}) values to those obtained on previous modeling studies on *L. monocytogenes*.

The secondary models provided good estimates of the growth parameters *L. innocua* in both products. Additionally, the validation results show that these models could provide reliable estimates for growth of *L. innocua* as a function of temperature. However, among these models, the Baranyi-Roberts model provided the closest estimation to observed data. Gompertz and Logistic models overestimated the parameters at temperatures 25°C and above.

CHAPTER IV

QUANTITATIVE MICROBIAL RISK ASSESSMENT FOR *LISTERIA*

***MONOCYTOGENES* ON FRESH-CUT ROMAINE LETTUCE AND FRESH-CUT**

CANTALOUPE

4.1 Introduction

In recent years, the occurrence of *L. monocytogenes* in fresh produce has gained interest because of their increased susceptibility to contamination by this pathogen (Garrido et al., 2010). The assessment of the risk caused by this pathogen is very important due to the high mortality rate of the illness (20 to 40%) and widespread nature of the pathogen in foods and the environment (Carrasco et al., 2010).

In 2003, the Food and Drug Administration (FDA) and the Center for Food Safety and Applied Nutrition (CFSAN) published a quantitative microbial risk assessment (QMRA) study of listeriosis for 23 categories of RTE products containing vegetables. In that study, vegetables were categorized as relatively low risk groups (<1 case/year). However, it also suggested that additional investigations for the subdivision of the vegetables category into several different groups were needed because of the high uncertainty caused by the diversity of the products. Since then, several relevant studies on QMRA for *E. coli* 0157:H7, *Salmonella* and *L. monocytogenes* in fresh produce were published (Franz et al., 2010; Tromp et al., 2010; Carrasco et al., 2010; Danyluk and Schaffner, 2011; Chen et al., 2013; Ding et al., 2013; Puerta-Gomez et al., 2013b; Sant'ana et al., 2014; Omac et al., 2015). However, to this date, there is no published study focused on the risk of listeriosis associated with fresh-cut romaine lettuce and

fresh-cut cantaloupe for the whole U.S. population. Scallan et al. (2011) reported that *L. monocytogenes* is one of the major reasons of foodborne deaths in the U.S. As a result, produce-specific QMRA studies are needed to have a better insight the impacts of decontamination methods, cross contamination and temperature abuse.

Hence, the objective of this study was to conduct quantitative risk assessments to evaluate the effectiveness of intervention and handling steps on the potential risk of illness of listeriosis associated with fresh-cut romaine lettuce and fresh-cut cantaloupe. Experimental growth data from a suitable surrogate were used in the development of the model.

4.2 Risk Assessment Methodology and Data Sources

4.2.1 Hazard Identification

Codex Alimentarius (1999) defined hazard identification as “the identification and biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.”

Listeriosis, caused by *L. monocytogenes*, is a rare disease but often leads to serious illnesses or even death. *L. monocytogenes* can grow in a wide temperature rate (-0.4 to 45°C), a wide pH range (4.39 – 9.4), and water activity as low as 0.92 (ICMSF, 1996). The incidence of listeriosis in the United States was 2.9 cases per 100,000 people for 2009 and 2011 (CDC, 2013). Painter et al. (2013) reported that *L. monocytogenes* causes around 1500 illnesses, 91% of which were hospitalized, and about 18% of hospitalizations resulted in death annually. Recently, a *L. monocytogenes* outbreak

associated with cantaloupe caused 32 deaths in 146 confirmed illnesses (Danyluk et al., 2014). Therefore, it is crucial to evaluate the safety measures at all stages in the food chain to prevent or reduce *L. monocytogenes* contamination in fresh produce.

4.2.2 Hazard Characterization

Hazard characterization is the evaluation of the unfavorable health effects linked with the identified hazard (Codex Alimentarius (1999). Hazard characterization is assessed with a dose – response relationship. Dose-response models consist of three major components, the pathogen, the environment, and the host (McLauchin et al., 2004).

Dose-response can be defined as the possibility that occurrence of adverse health effects in a specified category of costumers who are exposed to a certain level of pathogen and/or toxin. A biological end-point of dose response model can vary such as infection, morbidity, mortality, or specific diseases caused by *L. monocytogenes* (Brown and Springer, 2002). Two models are generally used to quantify the dose-response relationship; (1) the threshold model and (2) the non-threshold (single hit) model. While the threshold model assumes that bacterial cells can cause illness after they reach a certain population level, the non-threshold model implies that a single bacterium can cause illness. A threshold model of *L. monocytogenes* is not available for humans due to ethical concerns and the severity of listeriosis. As a result, a dose-response relationship is generally acquired from foodborne outbreaks, animal experiments, and surveillance data (FAO /WHO, 2008). Based on those data, several models were developed to calculate probability of illness associated with *L. monocytogenes*. Some of these models (Exponential, Beta-Poisson, and Weibull-Gamma) were evaluated by the FDA (2003),

which reported that all the models provided a close fit. In the current study, a Weibull-Gamma (W-G) model for probability of illness was used to estimate the infectivity of *L. monocytogenes*. The main assumption in W-G model is that every single cell has a very small, but finite probability of causing illness (Farber et al., 1996; Lindqvist and Westöö, 2000; FAO/WHO, 2008; Carrasco et al., 2010). This model is described by the following equation:

$$PI = 1 - [1 + (D^b)/\beta]^{-\alpha} \quad (4.1)$$

where, PI is the probability of illness for individuals exposed to a certain dose (D), D is the number of *L. monocytogenes* ingested (CFU/serving), b is a parameter which determines the shape of the dose-response curve, and α and β are the gamma distribution parameters which describe the heterogeneity of host or pathogen. The values of α , b , and β were determined from previous studies (Lindqvist and Westöö 2000; Carrasco et al., 2010).

Equation (4.1) assumes that the probability of infection for the whole population is the same. This approach may be faulty because obviously immunologically compromised populations are more susceptible to invasive listeriosis infection. Moreover, these populations represent 20% of total population in the USA (Bemrah et al. 1998). Therefore, two β values, one for high risk groups and one for low risk groups, were used. For both subpopulations, $\alpha = 0.25$ and $b = 2.14$, while $\beta = 10^{15.26}$ for low risk groups and $\beta = 10^{10.98}$ for high risk groups which include pregnant woman, elderly, and newborn (Bemrah et al., 1998; Carrasco et al., 2010).

In the present study, data on serving size of fresh-cut cantaloupe were collected from the studies published by Hoelzer et al. (2012a) and Chen et al. (2013), while information on serving size of fresh-cut romaine lettuce was taken from Carrasco et al. (2010) and Hoelzer et al. (2012b). For both products, serving size was defined by cumulative probability distributions. Therefore, the output of the exposure model was determined as the ingested dose in colony-forming units (CFU) for serving size for both products.

The annual illness cases associated with *L. monocytogenes* in the U.S. for each product were estimated as follows. First, the population of the U.S. (reported as 308,745,538 in 2010 by Howden and Meyer, 2011), was divided into two fractions according to their susceptibility. The first group included the high-risk individuals (pregnant women, the elderly, and children) and it was assumed to be 20 percent of the total population (Bemrah et al. 1998; Carrasco et al., 2010). Hence, 80 percent of the population consisted of the healthy intermediate age sub-population (low-risk group). Second, for each produce, consumption percentage data were collected from Hoelzer et al. (2012a), who estimated the U.S. consumption of romaine lettuce and cantaloupe as 9.81%, and 3.03%, respectively. Although these numbers covered both fresh and fresh-cut produce, no reduction was made in the numbers to obtain a more conservative risk output. Finally, per capita consumption of cantaloupe (3.87 kg, USDA, 2012) and romaine lettuce (3.49 kg, USDA, 2011) were established. Then, the total number of annual servings of fresh-cut romaine lettuce (Eq. 4.2 and 4.3) and fresh-cut cantaloupe (Eq. 4.4 and 4.5) were calculated as:

$$C = P * 0.0981 * 0.20 * 3490 \quad (4.2)$$

$$C = P * 0.0981 * 0.80 * 3490 \quad (4.3)$$

$$C = P * 0.0317 * 0.20 * 3870 \quad (4.4)$$

$$C = P * 0.0317 * 0.80 * 3870 \quad (4.5)$$

$$S_N = \frac{C}{S_S} \quad (4.6)$$

Herein, P is the population of the U.S.; S_N is the number of annual servings; C is the annual consumption (g) of each product consumed in the U.S.; and S_S is serving size (g). Annual consumption data were also calculated assuming the whole population was in the high risk group in order to observe and compare the differences between low and high risk estimations.

Next, the estimated cases of listeriosis for each population group per year were calculated as (Danyluk and Schaffner, 2011):

$$ECL = P(D) * S_N \quad (4.7)$$

Herein, ECL is the estimated number of cases of listeriosis.

4.2.3 Exposure Assessment

Exposure assessment is “the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food, as well as exposures from other sources if relevant” (Codex Alimentarius, 1999). The appropriate exposure model involves several areas such as pathogen prevalence and concentration in raw

produce, impact of the control measures during processing, distribution, handling, post-retail growth, and the amounts of food eaten. Since covering all these steps from farm to fork is impossible, assumptions, estimations, and models based on accurate and sufficient data are needed.

Fresh-cut romaine lettuce was assumed to be contaminated with *L. monocytogenes* during harvest by an unknown source. Between harvesting and consumption, a processing stage which includes disinfection treatments, packaging, storage and transportation, and display for sale at markets was applied. Because of the lack of data and difficulties in estimations, it was assumed that fresh-cut cantaloupe was contaminated with *L. monocytogenes* after washing treatments. Postharvest contamination and cross-contamination scenarios were estimated based on previous studies (Schaffner, 2004; Perez-Rodriguez et al., 2011; Ding et al., 2013). Since *L. innocua* was assumed as the surrogate for *L. monocytogenes*, the growth of *L. innocua* was determined by laboratory experiments and growth models described in Chapter III (Sections 3.4.3., 3.4.4). Additional information regarding the supply chains of each product is provided below.

4.2.3.1 Description of the Exposure Assessment for Fresh-Cut Romaine Lettuce

Romaine lettuce is one of the most consumed leafy green vegetables in the United States. More than 90 percent of total production is produced in California and Arizona. Since *L. monocytogenes* is ubiquitous in the environment, contamination sources may vary and it may occur through soil, insects, water, and animals. In the field, romaine lettuces are machine harvested. After harvesting, a cooling process is needed,

and this process is influenced by the outside temperature. Rediers et al. (2009) reported that endive needed 3 hours of cooling on a warm day (14-35°C), and 2 hours of cooling were needed on a moderate day (5-19°C) to cool endive to 10°C. Koseki and Isobe (2005b) also determined that the required cooling time for iceberg lettuce was 3 hours on moderate conditions. Romaine lettuces are then sent to the processing facility for washing (water and chlorine) treatment. After being minimally processed in the facility, fresh-cut romaine lettuce is transported to markets (Figure 4.1). Because the produce are packaged, it was assumed that there was no additional contamination point. Also, based on USFDA (2008) recommendations, it was assumed that produce were transported in refrigerated trucks between 0 and 5°C. Hence there was no temperature abuse during transportation. Temperature range is the most important factor in the entire process because the growth rate of *L. monocytogenes* is highly affected by temperature (Chapter III, 3.4.3).

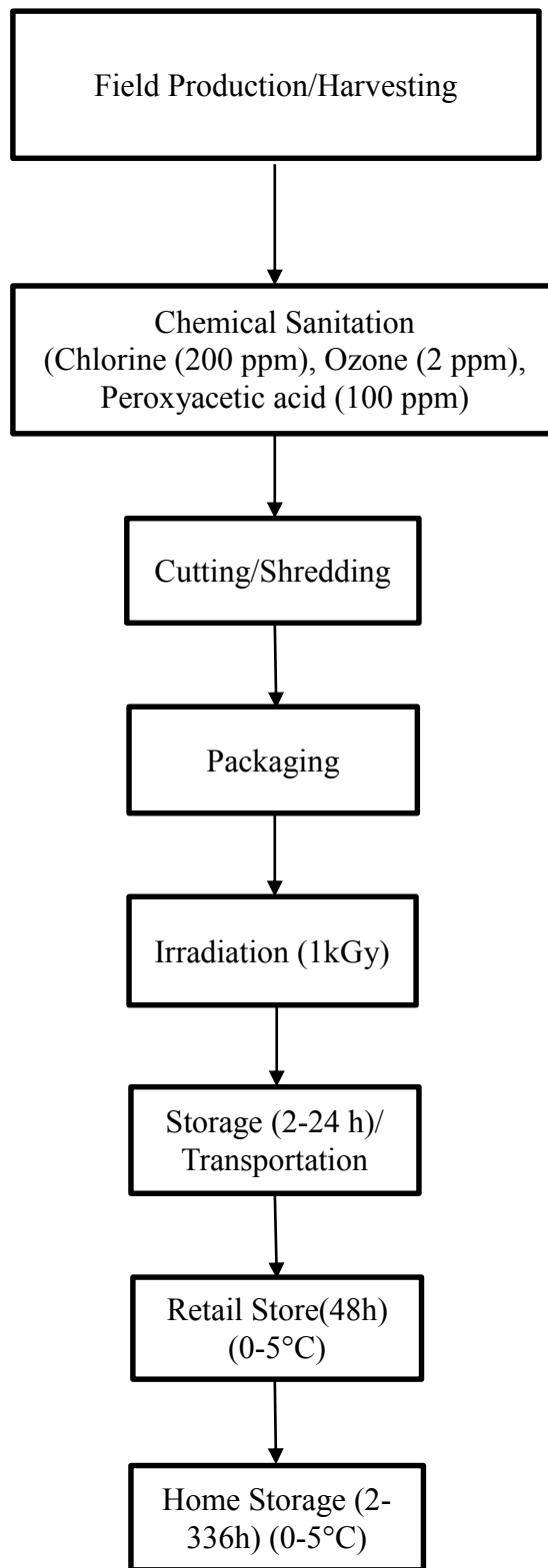


Figure 4.1 Flow chart of fresh-cut romaine lettuce.

Since time and temperature are critical parameters affecting microbial growth, they should be clearly defined. This study assumed that fresh-cut romaine lettuce had 14 days shelf-life at the temperature distribution used of the study (Section 4.2.3.8). Even though it was assumed that cold chain was maintained during transportation and display, sometimes temperature fluctuations can occur accidentally (Koseki and Isobe 2005b; Ding et al., 2013). The FDA recommends that display temperature should not be above 5°C (USFDA, 2010). However, fluctuations may occur during the display of fresh-cut produce (Jacxsens et al., 2002; Nunes et al. 2003; Koseki and Isobe 2005b; Ding et al., 2013). In the current study, temperature distribution described by Nunes et al. (2003) was used. In that study, the temperature in retail display for fresh-cut fruits and vegetables was 3.76°C with a standard deviation 0.89°C. The data were fitted to a normal distribution.

Several scenarios were prepared to evaluate the likely impact of different prevention steps, time, temperature abuse, and cross-contamination on fresh produce (Tables 4.1 and 4.4). Since fresh-cut cantaloupe and fresh-cut romaine lettuce have different post-harvest applications as described above, different scenarios were created and evaluated for each product.

4.2.3.2 Scenario Analysis of Fresh-Cut Romaine Lettuce

The first scenario **(1)** was called “baseline” which comprises standard procedures from harvesting to consumption without temperature abuse or cross-contamination. This scenario was also used for comparison with other scenarios to see the effects of the risk management options. The second scenario **(2)** comprises baseline plus irradiation

treatment (1 kGy, Mintier and Foley, 2005). The third scenario (3) was designed to investigate the impact of ozone treatment. In this scenario, baseline was created with ozone treatment (2 ppm) instead of chlorine (Olmez and Akbas, 2008). Effectiveness of alternative treatment methods were evaluated in the fourth (4) and fifth (5) scenarios. The sixth (6) scenario was the potential cross-contamination point during processing. In scenario seven (7), the effect of temperature abuse was evaluated. Product was left at room temperature (20°C) for 24 hours, instead of immediately placing it inside a domestic refrigerator. Lastly, the consumption time was set to 336 hours (14 days) to elucidate its impact on the potential risk (eighth (8) scenario.)

Table 4.1. ‘What if’ scenarios for fresh-cut romaine lettuce

Number	Scenario
1	Baseline
2	Baseline + Irradiation
3	Ozone
4	Peroxyacetic acid
5	Atmospheric plasma
6	Cross contamination
7	Temperature abuse (at home) – 20°C for 24 h
8	Consumption time (336 hours after packing)

4.2.3.3 Prevalence and Initial Level of *Listeria monocytogenes* in Fresh-Cut Romaine Lettuce

Initial contamination level of *L. monocytogenes* in the fresh products after harvest is determined by prevalence and initial level values. These values are essential to

create a baseline for QMRA. Contamination mechanism is not entirely clear although lettuce and cantaloupe can be contaminated with *L. monocytogenes* in many different ways, like contamination through soil, insects, processing environment, or handling mistakes (Beuchat, 1996).

There are extensive amount of surveys regarding the prevalence of *L. monocytogenes* in lettuce. Tang et al. (1994) reported that prevalence of *L. monocytogenes* in lettuce as 3.6% in Kuala Lumpur. Sant'ana et al. (2012a) showed that prevalence of *L. monocytogenes* in lettuce was 2% in Brazil. Ding et al. (2013) detected *L. monocytogenes* in 5.88% of lettuce samples in Korea. In the present study, data from 14 previous surveys were used to create a reliable prevalence input (Table 4.2). The prevalence data was described by using beta and pert distributions. The initial concentration shows the level of *L. monocytogenes* contamination in romaine lettuce after harvest (Table 4.3). This input was taken from Gombas et al. (2003) and described with a cumulative probability distribution. In that study, first, authors determined the prevalence of *L. monocytogenes* on bagged salads, then they determined the concentration of positive samples.

Table 4.2. Prevalence of *L. monocytogenes* in vegetables.

Source	Food	Number of Samples	Prevalence (%)	Number of positive samples
Gombas et al., 2003	Bagged salads	2966	0.74	22
Heisick et al., 1989b	Cabbage	92	1.1	1
Prazak et al., 2002	Cabbage	130	1.5	2
Lin et al., 1996	Vegetables salads	63	1.6	1
Velani and Roberts, 1991	Salad vegetables	108	1.8	2
Carrasco et al., 2010	Salad vegetables	263	2.3	6
Carrasco et al., 2010	Lettuce	28	3.6	1
FDA/CFSAN, 2003	Vegetables	9223	3.6	332
Legnani et al., 2004	Raw vegetables	43	6.9	3
Francis and O'Byrne 2006	Romaine Lettuce	80	11.8	9

To describe the effects of prevention methods, cross contamination, and temperature abuse, a relative growth rate was used (Tromp et al., 2010). Relative growth rate (RG in %) is described as the percent change from one point to another, and calculated as;

$$RG = ((y_t - y_0) * 100) / y_0 \quad (4.8)$$

Herein, y_0 is the initial concentration density (log CFU/g); and y_t is the concentration of the pathogen at time t (log CFU/g).

4.2.3.4 Washing and Sanitizing Treatments

Unlike fresh-cut cantaloupe, fresh-cut romaine lettuce can be washed after the cutting step. Moreover, when the sanitizing studies on fresh-cut romaine lettuce and whole lettuce were examined, there was little or no difference between the log reductions of *L. monocytogenes* populations. Furthermore, washing the lettuce right after cutting it may reduce the potential of cross-contamination from the cutting equipment. In the current study, washing treatment data for fresh-cut romaine lettuce were determined from actual experiments (Chapter III, 3.4.1.). The reduction in *L. monocytogenes* population was calculated as 1.55 log CFU/g and all the results were fitted to a normal distribution. Detailed explanation of the washing procedure and growth data results were provided in Chapter III (Sections 3.3.6 and 3.4.1, respectively).

Table 4.2. Prevalence of *L. monocytogenes* in vegetables (continued).

Source	Food	Number of Samples	Prevalence (%)	Number of positive samples
De Simon et al., 1992	Vegetable salads	103	7.8	8
Harvey and Gilmour, 1993	Raw vegetables	66	10.6	7
Olaimat and Holley, 2012	Radish	132	14.4	19
Arumugaswamy et al., 1994	Leafy vegetables	22	22.7	5
Heisick et al., 1989b	Radish	68	36.8	25
Sant'ana et al. 2012	Lettuce	152	1.97	3
Ding et al. 2013	Fresh-cut Lettuce	68	5.88	4
Szabo et al. 2000	Fresh-cut Lettuce	120	2.5	3

Table 4.3. Initial concentration of *L. monocytogenes* in lettuce (Adapted from Gombas et al., 2003).

Concentration (Log CFU/25 g)	Number of positive Samples	f(x) ^a	F(x) ^b
0-0.4	17	0.77	0.77
0.4-1.4	1	0.04	0.82
1.4-2.4	1	0.04	0.86
2.4-3.4	2	0.09	0.95
3.4-4.4	1	0.04	1

^af(x): prevalence $f(x) = i/n(+1)$ where 'i' is the number of positive samples and 'n' is the total number of samples

^bF(x): Cumulative frequency

4.2.3.5 Cross-Contamination

Fresh-produces are packaged after washing and sanitizing before being transported to retail stores. Cross-contamination is the biggest issue in this process as there are numerous surfaces such as conveyors, handling equipment, sorting tables, and containers in contact with the produce (Johnston et al., 2006; FAO/WHO, 2008). Cross-contamination is the transfer of bacteria from one food item to another by handling oversights, water washing, and packing equipment (Ding et al., 2013). Johnston et al. (2006) determined that the population of *E. coli* increased by 0.16 log CFU/g during the packaging of cabbage. The authors suggested that cross contamination was the most

possible explanation. The contamination loads of pathogens on various kinds of surfaces range from 2.12 to 7.43 log CFU/g (Chen et al., 2001). Moreover, FDA investigations on recent listeriosis outbreaks linked to cantaloupe, revealed that pathogens which caused the outbreaks existed in the packaging environment (USFDA, 2012; USFDA, 2013). These studies emphasize that, in the absence of hygiene and equipment sanitation rules, the possibility of cross-contamination increases. Several studies have predicted the onset of cross-contamination from different surfaces and different equipment with various mathematical models (Hoelzer et al., 2012c). However, Perez-Rodriguez et al. (2011) demonstrated that there was no agreement between these studies, and results may vary. In the current study, the effect of cross contamination on probability of illness, PI , (Eq. 4.1) was described using a uniform distribution from previous studies (Chen et al., 2001) followed by a transfer coefficient described by Ding et al. (2013). Transfer coefficient is the percent of cells transferred from one surface to another (Perez-Rodriguez et al., 2008). It is an empirical model that depends heavily on source, recipient, and number of observations (Hoelzer et al., 2012c).

4.2.3.6 Alternative Intervention Steps

Since thermal sanitation methods like pasteurization and sterilization are not applied to minimally processed fresh and fresh-cut products, and washing treatments have a limited effect on microbial quality, alternative methods have gained popularity in recent years (Gil et al., 2009). The current study assessed the effect of several intervention treatments, including irradiation, atmospheric plasma, ozone, and peroxyacetic acid on the probability of illness.

The impact of electron beam and gamma irradiation on reduction and decontamination of *L. monocytogenes* in different types of lettuce has been studied extensively (Niemira et al., 2002; Han et al., 2004; Mintier and Foley, 2005; Niemira, 2006; Niemira, 2007; Niemira, 2008). All these studies show that irradiation of romaine lettuce at 1 kGy can effectively reduce *L. monocytogenes* population. However, above 1 kGy, the quality parameters such as texture, flavor, and color were affected negatively from the treatment (Prakash et al., 2000; Han et al., 2004). In the current study, the effect of an e-beam irradiation step was evaluated from currently available experimental data (Mintier and Foley, 2005). According to that study, the D₁₀-value of *Listeria* spp. in fresh-cut romaine lettuce was 0.17 kGy and the survival of *L. monocytogenes* was calculated as:

$$S_{irr} = \frac{N}{N_0} = e^{-D/D_0} = e^{-2.303D/0.17} = e^{-13.54D} \quad (4.9)$$

herein, N_0 refers to the initial number of microorganisms (CFU/g), N refers to the number of remaining microorganisms (CFU/g) after exposure to dose D (in kGy), D_0 is the mean lethal dose (in kGy), or the dose required to reduce the survival fraction S to $1/e$ (i.e., 37%) and D_{10} is the radiation D-value or required dose for 90% reduction of the microbial population. In the present study, the radiation treatment was set up as exposure to 1.0 kGy at room temperature (~21°C) using an electron beam (Han et al., 2004).

Critzer et al. (2007) found that 3 min exposure of inoculated lettuce to 1 atm uniform glow discharge plasma resulted in 3 log reduction in the *L. monocytogenes* population. Peroxyacetic acid is another alternative treatment method approved by FDA.

Hellstrom et al. (2006) reported that 1.7 log CFU/g reduction was observed in the population of *L. monocytogenes* when 100 ppm peroxyacetic acid was applied to fresh-cut lettuce. Olmez and Akbas (2009) optimized ozone treatment for lettuce, and found that 2 min exposure of 2 ppm ozone resulted in 1.5 log CFU/g reduction, and was the optimal condition for maintaining product quality. All in all, all of these sanitizing methods are either equally or more efficient than chlorinated water. Table 4.3 shows the 'what if' scenarios related to these alternative treatments.

4.2.3.7 Time and Temperature Distribution of Fresh-Cut Romaine Lettuce

In the current study, the time and temperature distributions were examined under two different segments; (1) transportation and retail store conditions and (2) home storage. For the first segment, time distribution data were taken from Jacxsens et al. (2002). The time distribution was assumed to begin when the product left the processing facility and it ended with the actual purchase. Temperature distribution data were acquired from Nunes et al. (2003).

The time distribution of home storage data used in this study was reported by Danyluk and Schaffner (2011). Home temperature distribution information was taken from the study conducted by Pouillot et al. (2010). The effect of temperature and consumption time on the probability of illness (Eq. 4.1) was evaluated by creating “what if” scenarios (Section 4.3.1).

4.2.3.8 Growth of *Listeria monocytogenes* in Fresh-Cut Romaine Lettuce

The growth of *L. monocytogenes* in fresh-cut romaine lettuce was estimated by using the Baranyi model (Eqs. 2.3 and 2.4, Chapter II). Ratkowsky equation then was

used to define the model parameters as a function of temperature. (Eqs. 3.3 and 3.4, Chapter III).

4.2.3.9 Description of the Exposure Assessment for Fresh-Cut Cantaloupe

Cantaloupe is a crucial crop in Texas, harvested mostly in Uvalde County and the Rio Grande Valley. The climate of these areas have been determined as continental, semi-arid, and subtropical-sub humid. The range of temperatures in these areas is between 3-17°C in January and 22-37°C in July (Puerta-Gomez et al., 2013a).

Cantaloupes are sent to the processing facility for washing treatment. Castillo et al. (2009) emphasized that the washing treatment is generally applied in multiple steps. To prevent cross-contamination, in every step, fresh water should be applied in every step, and water quality parameters such as pH and organic load should be monitored.

Disinfectants, chlorine (100-200 ppm) and ozone (up to 5 ppm), are typically used in these steps. Disinfectant level should be monitored carefully. After that, a de-watering belt removes the wash water and sanitizers, and the produce. Figure 4.2 shows the flow chart of the cantaloupe processing and distribution steps. Cantaloupes are then stored and transported to retail stores in refrigerated trucks in order to limit bacterial growth. Fresh-cut fruits are usually stored from 0.5 days 12 days in at-home refrigerators, and generally consumed within 4 days (USFDA, 2003).

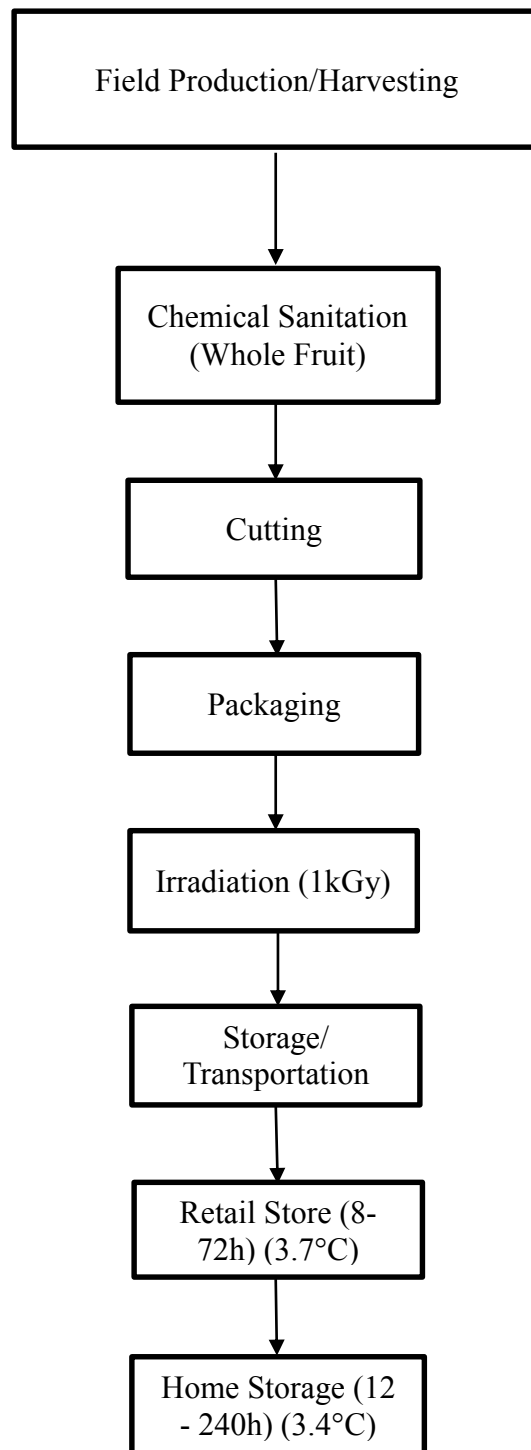


Figure 4.2 Flow chart of fresh-cut cantaloupe chain

4.2.3.10 Scenario Analysis for Fresh-Cut Cantaloupe

The first scenario (1) was similar to that considered for fresh-cut romaine lettuce; again, a baseline was created without temperature abuse and cross-contamination. The effect of irradiation treatment (1 kGy target dose) on the probability of illness (*PI*) was evaluated in the second (2) scenario (Rodriguez et al., 2006). Using current data from the literature, the third (3) scenario examined the effect of cross-contamination after the cutting step. The effect of temperature abuse at home was evaluated in the fourth scenario (4) for 24 hours at 20°C. Lastly, the fifth (5) scenario assessed the effect of time of consumption on the probability of illness. Unlike the case for fresh-cut romaine lettuce, the maximum consumption time was set as 240 hours (10 days) according to a study conducted by Chen et al. (2013).

Table 4.4. ‘What if’ scenarios for whole cantaloupe

Number	Scenario
1	Baseline
2	Irradiation
3	Cross contamination
4	Temperature abuse (home) (20 °C for 24 hours)
5	Consumption time (240 hours after packaging)

4.2.3.11 Prevalence and Initial Level of *Listeria monocytogenes* in Fresh-Cut Cantaloupe

Unlike the case for fresh-cut romaine lettuce, initial concentration and prevalence data regarding to *L. monocytogenes* for fresh-cut cantaloupe are very limited. Several studies on *Salmonella* (Castillo et al. 2004; Duffy et al. 2005; Espinoza-Medina et al. 2006) and *E. coli* (Castillo et al. 2004) were not used in the current study to determine the prevalence because of the concern that increased prevalence and the initial population of *L. monocytogenes* might result in an unrealistic increase in the risk of listeriosis. Therefore, prevalence and initial contamination data were obtained from Chen et al. (2013). In that study, five of 425 samples were found to be contaminated with *L. monocytogenes*. In addition, the initial concentration of *L. monocytogenes* in fresh-cut cantaloupe was determined by a normal distribution in which the mean was - 0.97 log CFU/g and standard deviation was 0.003 log CFU/g (Chen et al., 2013).

4.2.3.12 Washing and Sanitizing Treatments

In this study, the effect of washing and sanitizing on the reduction of *L. monocytogenes* population in fresh-cut cantaloupe was not evaluated. As mentioned in section 4.3.3.11, there are hardly any studies on prevalence and initial concentration of *L. monocytogenes* in fresh-cut cantaloupe, while there is no such study in whole cantaloupe. In industrial production of fresh-cut cantaloupe, the cutting process is carried out after washing the fruits (Figure 4.2). Although there are some studies on the internalization of pathogen into the fruit's flesh (Chimbombi et al. 2013) and possible cross-contamination of fresh-cut cantaloupe during cutting (Ukuku and Fett, 2002;

Ukuku et al. 2012; Vadlamudi et al. 2012), there is no reliable way to link the pathogen concentration in whole cantaloupe with that in fresh-cut cantaloupe because of limited data. Since the only prevalence and initial concentration data belong to fresh-cut cantaloupe, it was assumed that the product was already subjected to washing treatments.

4.2.3.13 Cross-Contamination of Fresh-Cut Cantaloupe

Pathogens most likely contaminate the cantaloupe flesh during the cutting practices (Beuchat, 1996). However, as the prevalence data used in this study was on fresh-cut product, it was assumed that even if cross-contamination occurred during the washing or cutting steps, contamination level was covered in initial concentration data. In the current study, the most likely cross contamination points were identified as the packaging and handling steps. In addition, it was assumed that there was no additional cross contamination points after production (i.e., during transportation and retail).

4.2.3.14 Irradiation of Fresh-Cut Cantaloupe

Since chemical sanitizing agents are always used on the whole cantaloupe, irradiation would be the only option to reduce the pathogen load on the fresh-cut produce. In this study, the effect of irradiation treatment on *L. monocytogenes* population was evaluated in scenario (2) using data from Rodriguez et al. (2006). According to that study, the D₁₀-value of *L. monocytogenes* was 0.15 kGy. Therefore, the survival of *L. monocytogenes* was calculated as:

$$S_{irr} = \frac{N}{N_0} = e^{-D/D_0} = e^{-2.303D/0.15} = e^{-15.35D} \quad (4.10)$$

Herein, N_0 refers to the initial number of microorganisms (CFU/g), N refers to the number of remaining microorganisms (CFU/g) after exposure to dose D (in kGy), D_0 is the mean lethal dose (in kGy), or the dose required to reduce the survival fraction S to $1/e$ (i.e., 37%) and D_{10} is the radiation D-value or required dose for 90% reduction of the microbial population; a value of D equal to 1 kGy was selected because irradiation at this dose level does not affect the quality of the fruit (Castell-Perez et al., 2004).

4.2.3.15 Time and Temperature Distribution of Fresh-Cut Cantaloupe

Similar to the study on romaine lettuce, the time and temperature distributions started right after the product left the facility. For the transportation step, data were taken from Puerta-Gomez et al. (2013b) and subjected to uniform distribution. Temperature distribution in the retail step was obtained from Nunes et al. (2003).

Domestic refrigerator temperature distribution was assumed as 3.4°C with a standard deviation of 2.4°C (Chen et al., 2013). Home storage time reported by Chen et al. (2013) was at least 0.5 day with a maximum of 10 days. These data were subjected to a uniform distribution to create an even time profile. Like romaine lettuce, the effect of temperature abuse and consumption time on the probability of illness (PI) was evaluated in scenarios (4) and (5) respectively.

4.4 Risk Characterization

Risk characterization was defined by Codex Alimentarius (1999) as; “the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard

characterization, and exposure assessment.” In other words, risk characterization is a combination of hazard identification, exposure assessment, and hazard characterization. In the current study, after the population of *L. monocytogenes* in fresh-cut romaine lettuce and fresh-cut cantaloupe was evaluated with the exposure assessment, Weibull-Gamma dose-response model was used to obtain the risk characterization part of risk assessment. Different scenarios were built based on assumptions and previous studies, and the QMRA was created in an Excel (Microsoft, Redmond, WA) spreadsheet to evaluate the risk by using Monte Carlo simulation.

4.5 Monte Carlo Simulation

The Monte Carlo simulation, an alternative to analytic techniques, expresses a powerful and accurate method for including both the stochastic and epistemic uncertainty of a problem (Hald et al., 2004). A single point is spontaneously chosen from each of the likelihood distributions assigned to each input parameter including epistemic uncertainty in Monte Carlo simulation. These spontaneously chosen single points are then used to compute a mathematical solution, as described by the risk assessment model. Several software programs use this simulation to achieve stochastic models which are impossible to solve analytically. Stochastic model, which is described as a probability distribution of possible values, provides all the information available for each input variable (Vose, 2000). In this study, each risk assessment model was simulated three times using the @RISK software (Palisade Corp. New Field, NY) with 10,000 iterations.

4.6 Results and Discussion

4.6.1 Quantitative Risk Assessment for Fresh-Cut Romaine Lettuce

In scenario (1), the median prevalence of *L. monocytogenes* on fresh-cut romaine lettuce was 11%, while the minimum and maximum values were 3% and 25% respectively (Figure 4.3). Median of initial concentration was estimated -0.1 log CFU/g, while the maximum value was as high as 5 log CFU/g (Table 4.5). Although these distributions were left-skewed, these results showed that in rare occasions, *L. monocytogenes* level on fresh-cut romaine lettuce would be very high. After the washing treatment, pathogen concentration was -1.65 log CFU/g which means that the population of *L. monocytogenes* in fresh-cut romaine lettuce was decreased by 97% with sanitizing treatments (Eq. 4.5). The simulation indicated that the temperature at retail store was between 0.2°C and 7.1°C. Although the temperature fluctuation was high, the median time in retail store was estimated has 54 hours. Considering the fact that lag time (t_{lag}) of *L. monocytogenes* is very high at 5°C (Chapter III, Table 3.2), pathogen level is constant at this stage. However, these results also showed that there would be enough time for the pathogen population to reach 7-8 log CFU/g level in the case of temperature abuse.

After data taken from Danyluk and Schaffner (2011) distributed, in the home storage stage of risk assessment, the time range before last consumption was found between 73 hours and 340 hours with a mean of 189 hours (Table 4.5). According to this distribution, on average, fresh-cut romaine lettuce is consumed 6 days before the end of shelf-life.

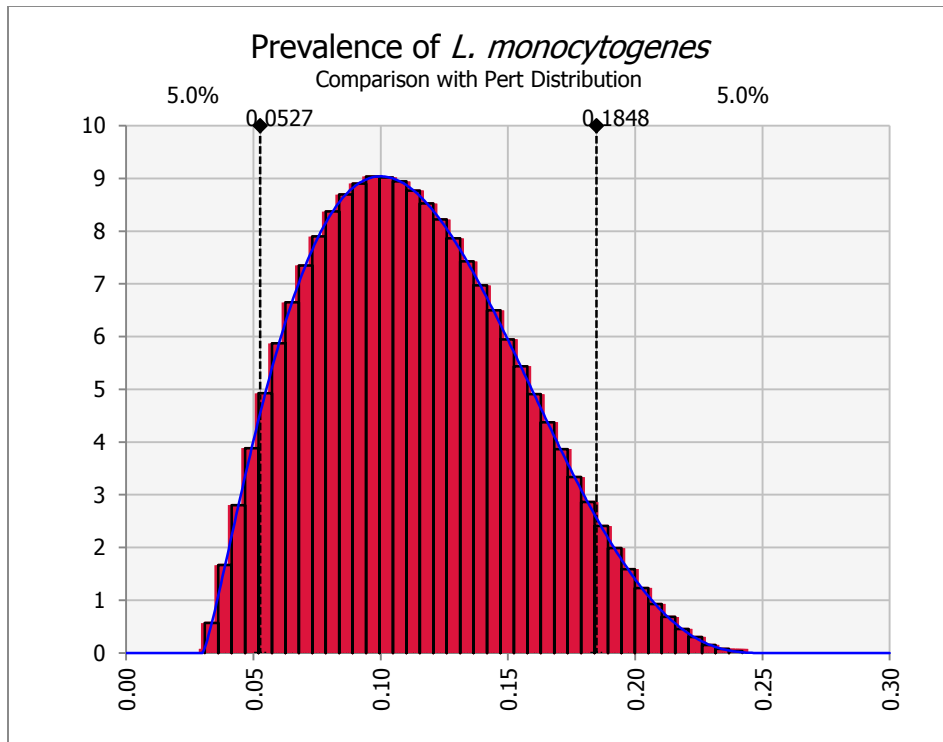


Figure 4.3. Probability distribution of the prevalence of *L. monocytogenes* on fresh-cut romaine lettuce.

Growth of *L. monocytogenes* on the leafy green was simulated using the experimental data presented in Chapter III. When combined with the temperature distribution, the median growth rate was 0.022 log CFU/g/h, which means that on average, the growth rate of *L. monocytogenes* was 0.52 log CFU/g per day (Table 4.5). This relatively low increase in the bacterial population could be attributed to low temperature distribution. Equation (3.8) yielded a lag time (t_{lag}) 99 hours for *L. monocytogenes*. As mentioned in Chapter III, growth rate and lag time was heavily dependent on temperature. After the growth model was combined with initial concentration, time, and temperature distributions, pathogen population at time of

consumption was 0.5 CFU/g. According to Eq. (4.8), the relative growth of *L. monocytogenes* in fresh-cut romaine lettuce was 36.7%. This result showed that if time and temperature could be maintained well during the product chain, the pathogen population would be very low at the point of consumption. According to serving size distribution, minimum and maximum consumption was 25g and 150g respectively, while the average consumption was 48.25g. Additionally, the cumulative distribution was left-skewed, which suggests that consumption rates are generally low. When serving size data was combined with the final number of pathogen population, it was found that pathogen level per serving was 25 CFU.

For each subpopulation, probability of illness values (*PI*) were calculated using Eq. (4.1). The *PI* values for susceptible and healthy populations were 2.69×10^{-9} and 1.41×10^{-13} , respectively. When these data were combined with information on prevalence, the actual exposure probability was 3.5×10^{-10} and 1.65×10^{-14} for susceptible and healthy populations, respectively. The cumulative frequencies of log probability of illness for both subpopulations are presented in Figure 4.4. The probability of illness values were surprisingly low which suggest that, unless cross-contamination or temperature abuse occur, common washing practices might be sufficient for controlling the pathogen in fresh-cut romaine lettuce. Indeed, after *PI* was multiplied by the population (Eq. 4.7) even when the entire population was considered as susceptible, estimated annual illness was 0.4 cases per year. Similar to a current study, FDA/CFSAN (2003) reported that predicted median cases of listeriosis for total U.S. population consuming vegetables on per annum are 0.2.

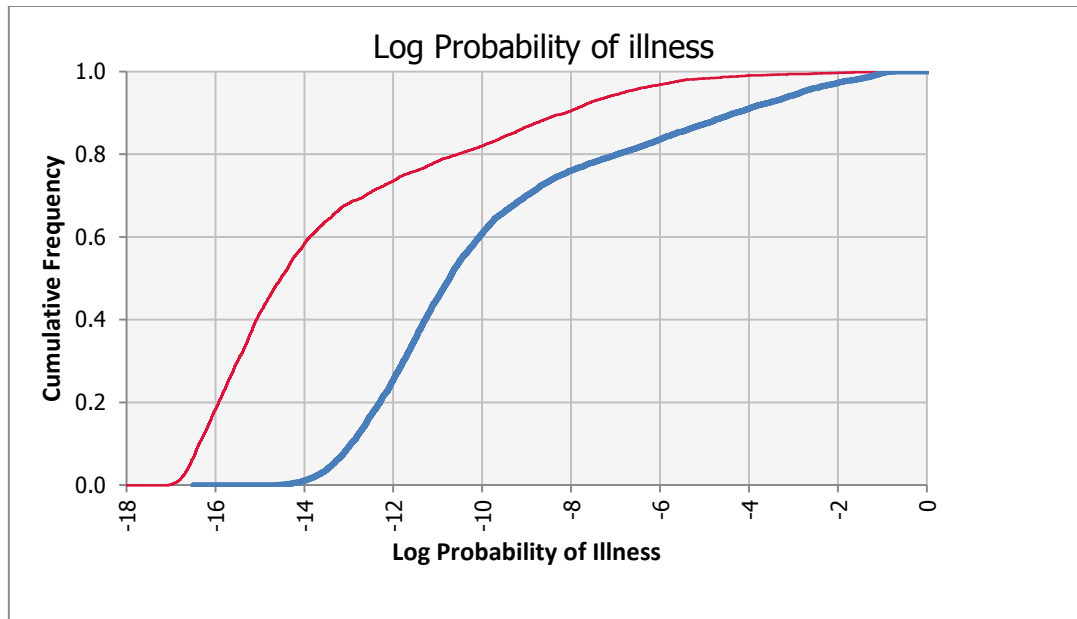


Figure 4.4. Log probability of illness (PI) for both subpopulations, Scenario (1)
 Red line: Healthy Population
 Blue line: Susceptible population

As mentioned in Section 4.3.3.6, on average, the washing treatment was less effective than other sanitizing methods (irradiation, peroxyacetic acid, and ozone) evaluated in this study, expected annual illnesses were less than 1 in scenarios (2)-(5). Among those scenarios, irradiation had the biggest impact on *L. monocytogenes* population (Scenario (2)). According to Eq. (4.8), the population of *L. monocytogenes* was reduced by 99.999% after exposure to ionizing radiation (1 kGy at room temperature). Even at the time of consumption, the relative growth of *L. monocytogenes* was -99.995%. Similarly, results were obtained in a QMRA for *L. monocytogenes* in baby spinach leaves showed that *L. monocytogenes* population at the time of consumption was 99.99% less when produce was irradiated (Omac et al., 2015).

Table 4.5. Model parameters and calculated values for eight scenarios of the probability of illness in fresh-cut romaine lettuce.

Model Parameter	Scenario #							
	1	2	3	4	5	6	7	8
Water washing (log CFU/g)	0.58	0.58	-	-	-	0.58	0.58	0.58
Chlorine washing (log CFU/g)	0.974	0.974	-	-	-	0.97	0.974	0.974
Cross-contamination (log CFU/g)	0	0	0	0	0	4.77	0	0
Irradiation dose (kGy)	0	1	0	0	0	0	0	0
Temperature abuse (°C, hours)	0	0	0	0	0	0	20°C, 24 h	0
Home Storage Temperature (°C)	4.44	4.44	4.44	4.44	4.44	4.44	4.44	4.44
Time (hours)	189	189	189	189	189	189	189	336
<i>L. monocytogenes</i> concentration at consumption (CFU/g)	0.53	1.5x10 ⁻⁵	0.67	0.38	0.02	0.63	0.83	11.74
Log probability of illness (Susceptible)	-10.7	-12.36	-10.56	-11.05	-12.04	-10.64	-9.6	-8.09
Log probability of illness (healthy)	-14.52	-16.63	-14.61	-14.91	-15.29	-14.53	-13.94	-12.18

After the irradiation step, cold atmospheric plasma was the second best treatment which reduced the *L. monocytogenes* population by 99.08% (Scenario (5)). At time of consumption, the level of *L. monocytogenes* was 91.93% less than the initial concentration. Unlike these two treatments, the effectiveness of ozone (Scenario (3)) and peroxyacetic acid (Scenario 4) was similar to the effect of using chlorinated water.

After ozone treatment, the relative growth rate was s -77%. In fact, pathogen population at the time of consumption was 28% higher than baseline. However, this difference did not affect expected annual illness value (Table 4.5). These results highlight that at the recommended dose (2 ppm) ozone might not be a good alternative to chlorinated water. In terms of food safety, increasing the exposure time or dose might increase the effectiveness of the treatment. On the other hand, peroxyacetic acid was more effective than chlorinated water and ozone treatments. Relative growth rate after peroxyacetic acid treatment was -87%. However, Kitis (2004) reported that peroxyacetic acid was an expensive compound. Furthermore, peroxyacetic acid shares similar disadvantages to other chemical sanitizers such as chlorinated water. These sanitizers are surface decontaminants, and offer no solution against internalized pathogens. When all treatment scenarios are compared, it can be argued that irradiation of fresh-cut romaine lettuce at 1 kGy is the most effective option in terms of food safety.

Cross-contamination (Scenario (6)) was not a critical issue for the safety of fresh-cut romaine lettuce. A possible explanation is that the transfer coefficient was very low (0.002), so even when contamination occurred, there was only 0.1 CFU/g increase in the population of *L. monocytogenes* at the time of consumption. At the time of consumption,

the pathogen level (0.63 CFU/g) was nearly identical to the baseline. As a result, no change was observed on the number of expected annual illnesses. However, it should be considered that the transfer coefficient is empirical, and may not cover the rare cases in which contamination might be very high (Perez-Rodriguez et al., 2011).

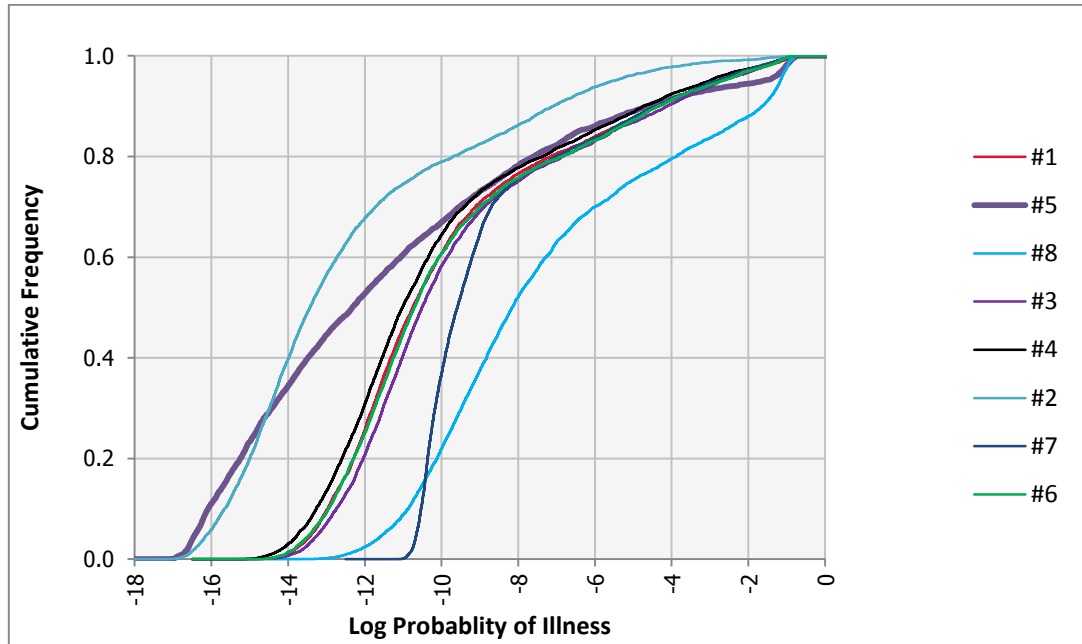


Figure 4.5. Log probability of illness for scenarios #1- #8 for susceptible subpopulation

- #1: Baseline
- #2: Irradiation
- #3: Ozone
- #4: Peroxyacetic acid
- #5: Cold Atmospheric plasma
- #6: Cross contamination
- #7: Temperature abuse
- #8: Consumption time (336 hours)

Temperature abuse made a major impact on expected annual illness numbers. A rapid increase was observed in *L. monocytogenes* population during the temperature abuse scenario. Compared to the baseline scenario (**1**), the level of *L. monocytogenes* at

time of consumption was 56% higher. In addition, the growth rate increased from 0.022 log CFU/g/h to 0.148 log CFU/g/h. As a result, the median of expected annual illness for the susceptible population was around 6 cases. A possible explanation is that lag time and growth rate are highly temperature-dependent, and at higher temperatures, these parameters would be higher.

Surprisingly, consuming fresh-cut romaine lettuce at the end of the shelf-life caused the highest annual illness numbers (Scenario **(8)**). The median of expected annual illness was 29 cases. Moreover, with the assumption of the entire population being susceptible to illness, the expectation increased to 128 annual cases. Correspondingly, the highest relative growth (442%) was observed in this scenario. These results can be explained by the high lag time of around 100 hours, as long as the temperature distribution was not altered (scenario **(7)**). As explained above, the mean consumption time was 189 hours. It can be argued that because of the long lag time, the pathogen level was steady for more than half of the consumption time. After that, due to a low temperature distribution, the grow rate was very limited, and did not have an impact on the pathogen population at the time of consumption. However, when consumption time increased to 336 hours (14 days or end of shelf-life), this allowed for the pathogen level to increase to very high values. These results verified that consumption time is one most important parameters regarding the safety of the leafy green.

4.6.2 Quantitative Risk Assessment for Fresh-Cut Cantaloupe

When both produces were compared, on average, prevalence of *L. monocytogenes* on fresh-cut cantaloupe (1.3%) was almost 10 times lower than on fresh-

cut romaine lettuce (11%). In a similar fashion, the mean initial concentration of *L. monocytogenes* on fresh-cut cantaloupe (0.1 CFU/g) was about 20 times lower than on fresh-cut romaine lettuce (2.16 CFU/g) (Figure 4.6).

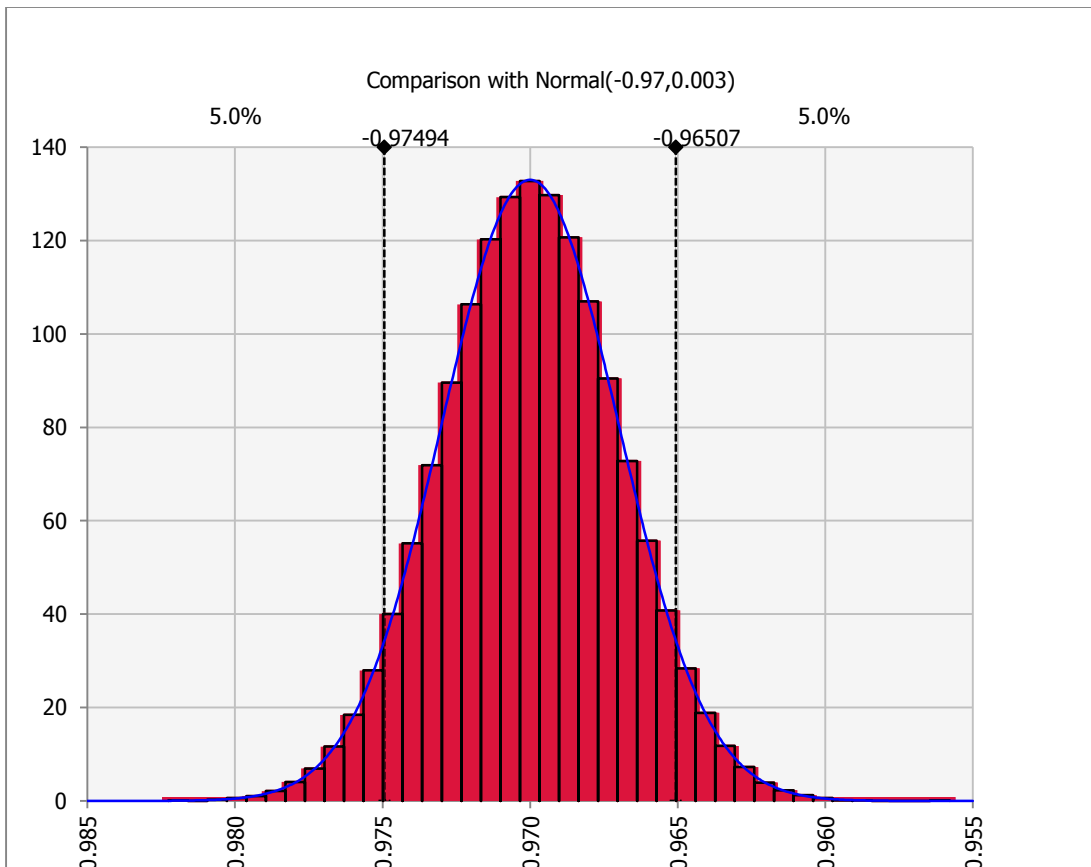


Figure 4.6. Probability distribution of the initial concentration of *L. monocytogenes* on fresh-cut cantaloupe.

Unlike fresh-cut romaine lettuce, the shelf-life of fresh-cut cantaloupe was 10 days at refrigerated temperature. However, according to the time distribution, on

average, fresh-cut cantaloupe was consumed after 5 days from leaving the processing facility. Temperature distribution yielded a growth rate of *L. monocytogenes* on fresh-cut cantaloupe as 0.048 log CFU/g/h, approximately 118% higher than on fresh-cut romaine lettuce. As stated in Chapter III, higher sugar content and milder pH values made the fresh-cut cantaloupe a better medium for the growth of pathogen. Furthermore, lag time was 87 hours, suggesting that pathogen levels on produce were stable throughout that period.

Pathogen level at the time of consumption was 10.72 CFU/g, about 20 times greater than on fresh-cut romaine lettuce (0.5 CFU/g). Due to the high growth rate, the relative growth of *L. monocytogenes* population was 9905% at the point of consumption (Eq. 4.8). Combined with the median serving size, the relative growth of *L. monocytogenes* population was 112.5g, and pathogen level per serving was 1206 CFU/g. The *PI* values (Eq. (4.1)) for the susceptible and healthy populations were 1.03×10^{-5} and 5.40×10^{-10} , respectively (Table 4.6). These findings confirm that the probability of illness is highly affected by the type of produce. For example, the *PI* associated with susceptible subpopulation of romaine lettuce consumers (2.69×10^{-9}) was close to the healthy subpopulation of fresh-cut cantaloupe consumers. The expected annual illness for the baseline was 17 cases (Table 4.6). When the entire population was assumed as susceptible, the expected case number increased to 69. Although the prevalence, initial concentration and consumption time were lower than for fresh-cut romaine lettuce, expected cases per year increased to 17. These results indicate that the medium (ie, produce type) had a huge impact on the risk of illness associated with *L. monocytogenes*.

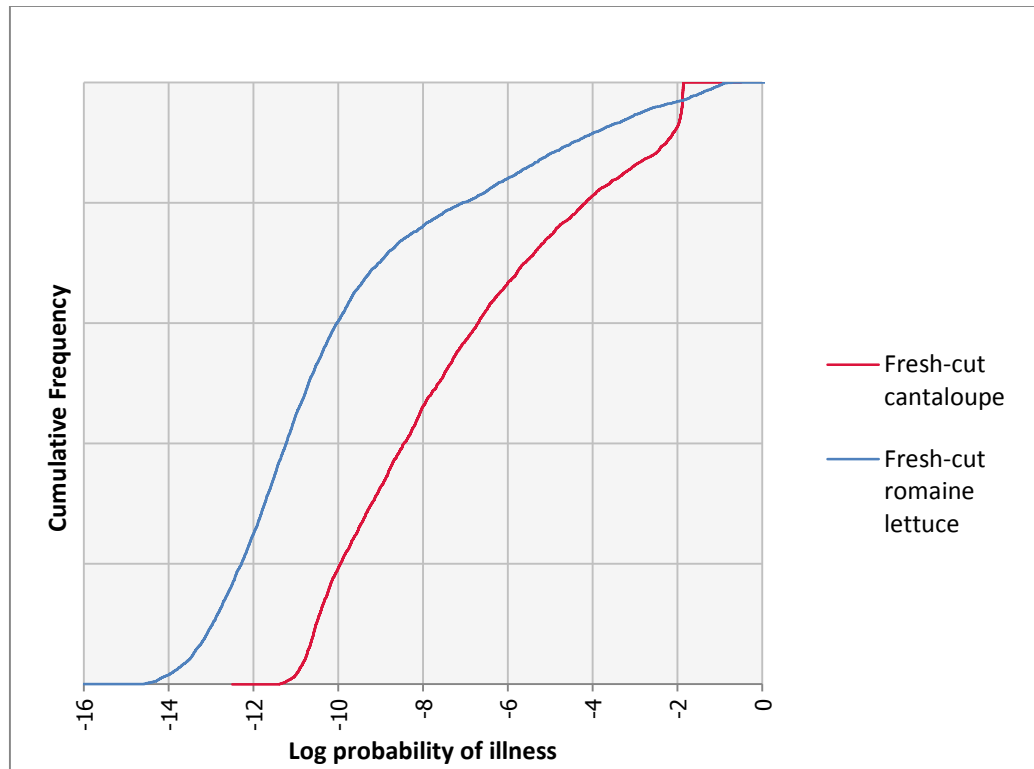


Figure 4.7. Comparison of log probability of illness for the baseline (#1) of both produce

Irradiation was the only prevention step evaluated in this study because of the assumption mentioned in Section 4.2.3.12. Implementation of an irradiation step reduced the pathogen population to more than 99.99%. Even at the time of consumption, the relative growth of *L. monocytogenes* was -99.75%. As a result, for all subpopulations, the number of expected cases per year was less than one. Considering the effect of irradiation on fresh-cut romaine lettuce, in terms of food safety, irradiation is probably the best solution available to reduce the onset of a breakout due to consumption of *Listeria*-contaminated items.

Table 4.6. Model parameters and calculated values for five scenarios of the probability of illness in fresh-cut cantaloupe.

Parameters	Scenarios #				
	1	2	3	4	5
Initial concentration of <i>L. monocytogenes</i> (log CFU/g)	-0.97	-0.97	-0.97	-0.97	-0.97
Cross-contamination levels (CFU/g)	0	0	0.002	0	0
Irradiation dose (kGy)	0	1	0	0	0
Temperature abuse (°C & hours)	0	0	0	20°C, 24h	0
Median Home Storage Temperature (°C)	3.4	3.4	3.4	3.4	3.4
Median Time (hours)	140	140	140	140	240
<i>L. monocytogenes</i> concentration at consumption (CFU/g)	10.72	2*10 ⁻⁴	10.75	42.77	258
Log probability of illness (Susceptible)	-6.86	-12.7	-10.56	-3.91	-12.04
Log probability of illness (healthy)	-11.1	-16.8	-14.61	-8.18	-15.29
Estimated Annual Case	17	<1	68	54	6685

Unlike the case of fresh-cut romaine lettuce, cross contamination had a big impact on the risk for foodborne illness from consumption of fresh-cut cantaloupe.

Although the assumed transfer coefficients were the same as with romaine lettuce, the number of expected cases per year increased to 68. Likewise, when the entire population was assumed to be immunologically compromised, the expected number increased to 223 cases. The pathogen level also increased from 0.107 CFU/g to 0.109 CFU/g with cross-contamination. This result shows that even an increase on pathogen level of 0.002 CFU/g can impact public safety. Furthermore, it can be said that the magnitude of the effect of cross-contamination highly depends on the type of produce. In terms of risk assessment, it is crucial to understand the growth patterns of pathogen on specific products.

Temperature abuse also caused a big increase in expected annual illnesses. The number of expected cases per year was 54 for the entire population and 220 for the susceptible population. Although the growth rate increased to 0.38 log CFU/g/h from 0.053 log CFU/g/h in a 24 hours period, the effect of temperature abuse was almost the same as cross-contamination. This result highlights the importance of cross-contamination as a critical issue regarding the onset of foodborne illness due to consumption of *Listeria*-contaminated fresh-cantaloupe. Preventing the cross contamination should a priority target for producers.

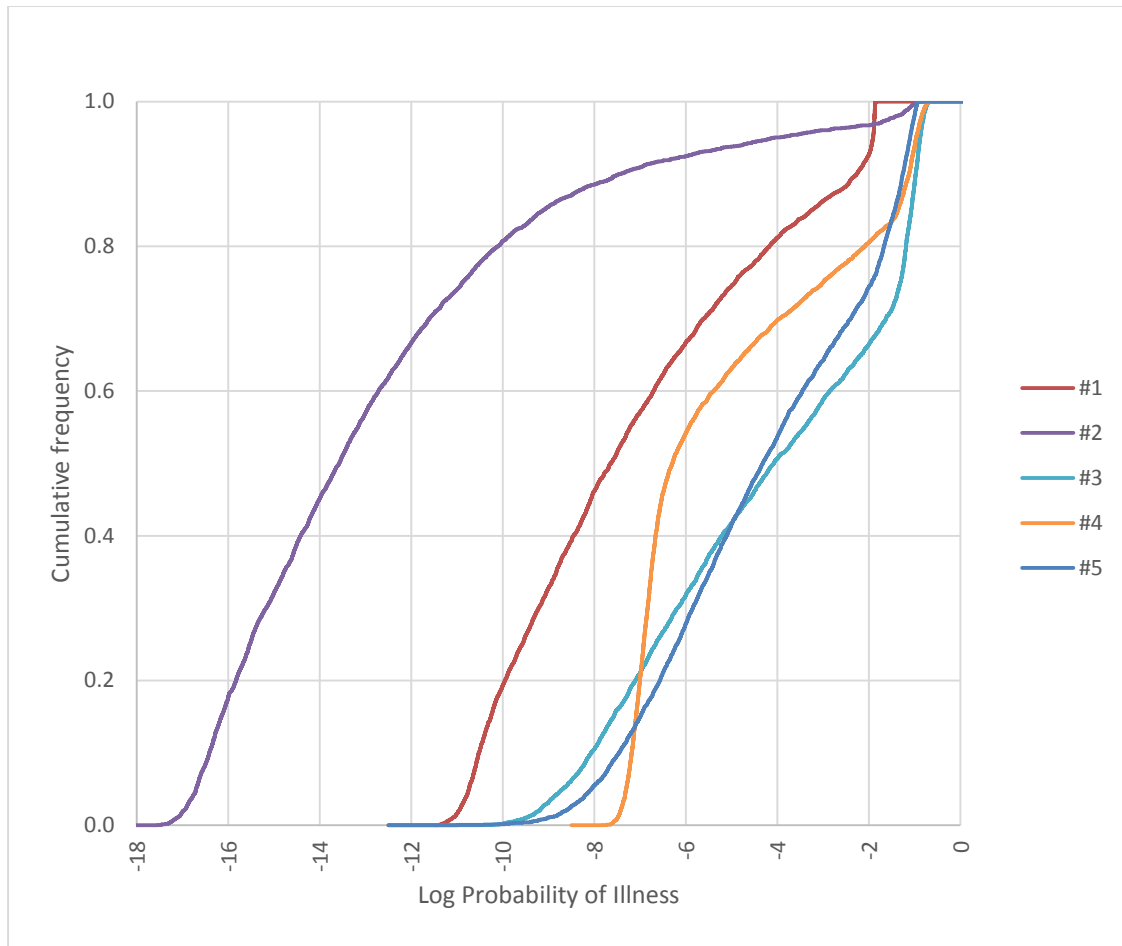


Figure 4.8. Log probability of illness for scenarios (1)- (5) for a susceptible subpopulation

- #1: Baseline
- #2: Irradiation
- #3: Cross contamination
- #4: Temperature abuse
- #5: Consumption time (336 hours or 14 days)

Like in the case of fresh-cut romaine lettuce, the highest increase in annual cases occurred when the consumption time was set to 240 hours (10 days). Simulation results showed that at the time of consumption pathogen population was 2313% higher than the baseline. Consequently, the number of cases of expected illnesses per years was 6685

and 31000 for general and susceptible populations, respectively. These results confirm that *L. monocytogenes* could pose a serious problem in fresh produce even under refrigeration temperatures. Although consumption time was not in conjunction with temperature abuse or cross-contamination, expected annual case numbers were more than 100 times higher than any other scenarios used in this study. It can be argued that apart from the initial pathogen concentration, time is singlehandedly the most important factor on the risk of listeriosis. This scenario may vary for other common pathogens like *Salmonella* as they do not grow as well under refrigeration temperatures.

4.7 Summary

This study showed that the quantitative risk assessment model can be used to evaluate the effect of intervention steps on the prevention of listeriosis due to consumption of fresh produce. The chemical treatments had similar low impact on pathogen load reduction, which suggests that other alternative treatments such as irradiation should be implemented. Irradiation was the most effective means to reduce the pathogen level on both fresh-cut produces because the estimated annual cases of listeriosis were reduced by more than 99% when this step was added to a typical fresh-cut produce processing and distribution chain. This study also showed that cold atmospheric plasma might be a good alternative treatment of fresh-cut romaine lettuce to reduce the potential of illness associated with *Listeria*.

Although the prevalence (10 times) and initial concentration (20 times) were lower in fresh-cut cantaloupe, the risk of illness associated with *L. monocytogenes* was 40 times higher than fresh-cut romaine lettuce. Moreover, cross contamination in fresh-

cut cantaloupe increased the expected annual illness to 68 cases. These findings demonstrate that pathogen growth rate is more critical than prevalence and initial pathogen concentration. When temperature abuse occurred at 20°C, the risk of listeriosis due to consumption of both produces increased. Therefore, in the summer season, the effect of temperature abuse can be dramatically higher. As a result, temperature distribution should be monitored closely in the production and retail stages, and the produce should be kept in the refrigerator at home. However, consumption time scenarios revealed that even when produce stored in refrigerated temperature, the risk of listeriosis increased with time. Indeed, the ability of *L. monocytogenes* to grow at lower temperatures makes the pathogen is a serious problem. The current study showed that preventing initial contamination, and implementing irradiation would be most effective options on reducing the risk of listeriosis associated with fresh-cut produce.

CHAPTER V

CONCLUSIONS

This research focused on the quantitative microbial risk assessment of illness from *Listeria monocytogenes* due to consumption of fresh-cut romaine lettuce and cantaloupe. In terms of washing the produce with chlorinated water (200 ppm) was more effective ($p < 0.05$) than washing only with water. In addition, the effect of washing treatments was more significant ($p < 0.05$) on fresh-cut romaine lettuce than on fresh-cut cantaloupe.

Experimental data on *Listeria innocua* were used to obtain growth curves at different temperatures (5-36°C). As expected, the growth of *L. innocua* was highly affected ($p < 0.05$) by temperature. Three different primary growth models were evaluated for goodness of fit. The maximum growth rate of *L. innocua* on fresh-cut cantaloupe was significantly higher than on fresh-cut romaine lettuce at 5 °C and 10°C ($p < 0.05$). Lag time was not observed at temperatures of 10°C and above. All the primary models provided accurate descriptions for maximum growth rate and maximum population density of *L. innocua*. However, at higher temperatures, the Gompertz and Logistic models overestimated these parameters. Because the suitability of the surrogate was validated by Omac et al. (2015) and the lack of access to BSL2 facilities, the growth patterns of the surrogate were used to predict the growth of the pathogen in the risk assessment study.

The second part of this research dealt with the quantitative microbial risk assessment (QRAM) for growth of *L. monocytogenes* in both produces. Growth data

from the surrogate served as input for the QRA model. The risk of illness per year associated with *L. monocytogenes* on fresh-cut cantaloupe (around 17) was significantly higher than on fresh-cut romaine lettuce (around 0.4) because cantaloupe is a more suitable media for the pathogen.

Cross-contamination and temperature abuse throughout the processing and distribution chain increased the risk of illness due to consumption of fresh-cut cantaloupe by altering the concentration and growth rate of the pathogen in the fruit. Consumption time was the most important risk factor for both types of produce.

When temperature abuse occurred at 20°C, the risk of listeriosis due to consumption of both produces increased. Therefore, in the summer season, the effect of temperature abuse can be dramatically higher. As a result, temperature distribution should be monitored closely in the production and retail stages, and the produce should be kept in the refrigerator at home.

Although the prevalence (10 times) and initial concentration (20 times) were lower in fresh-cut cantaloupe, the risk of illness associated with *L. monocytogenes* was 40 times higher. Moreover, cross contamination in fresh-cut cantaloupe increased the expected annual illness to 68 cases. These findings demonstrate that pathogen growth rate is more critical than prevalence and initial pathogen concentration.

In summary, fresh-cut produce should be periodically controlled in the processing system to prevent cross-contamination. Furthermore, the records of time and temperature should be regularly kept during the farm to table chain to monitor temperature abuse.

CHAPTER VI

RECOMMENDATIONS FOR FURTHER STUDY

Recommendations for future research focus on validation of *Listeria innocua* as a surrogate for *L. monocytogenes* in several types of produce, and the quantitative risk assessment for *L. monocytogenes* in fresh-cut produce. Therefore, it is recommended to:

- Test *L. innocua* under different environmental conditions (i.e. temperature) and in several commodities to establish its suitability as a surrogate for *L. monocytogenes*.
- Increase the accuracy of the dynamic models by including all parameters which affect the growth of *L. innocua* and *L. monocytogenes* in leafy green vegetables.
- Collect data regarding prevalence and initial concentration of *L. monocytogenes* in fresh-cut cantaloupe and fresh-cut romaine lettuce.
- Conduct surveys regarding retail and home storage time and temperature.
- Develop a cross-contamination model to describe the effect of cutting process on the transfer of *L. monocytogenes* in fresh-cut cantaloupe and fresh-cut romaine lettuce and other commodities.
- Collect data regarding consumption of each commodity by subpopulation in the U.S.

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APPENDIX A

Figure A.1. The observed growth of *Listeria innocua* on fresh-cut romaine lettuce at 5°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

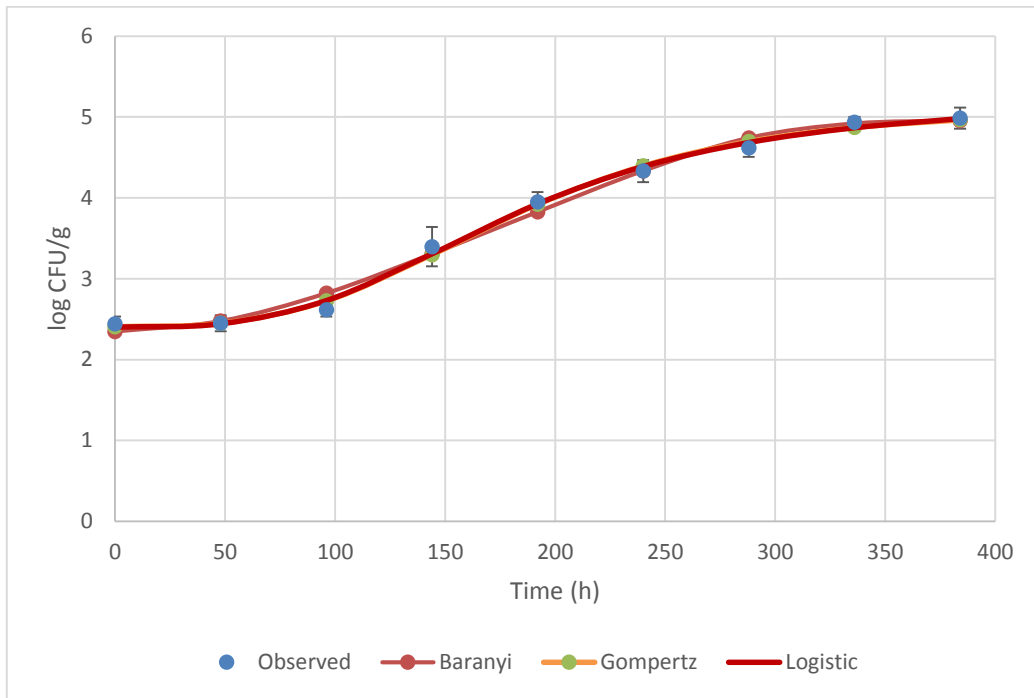


Figure A.2. The observed growth of *Listeria innocua* on fresh-cut cantaloupe at 5°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

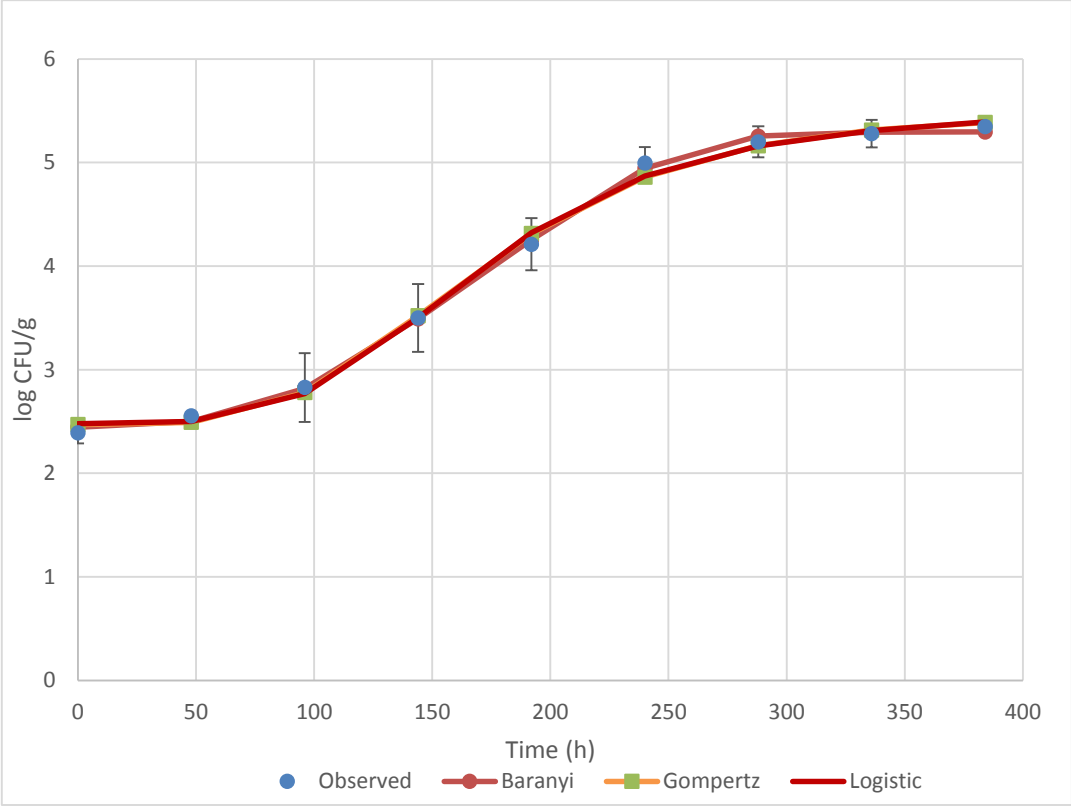


Figure A.3. The observed growth of *Listeria innocua* on fresh-cut romaine lettuce at 10°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

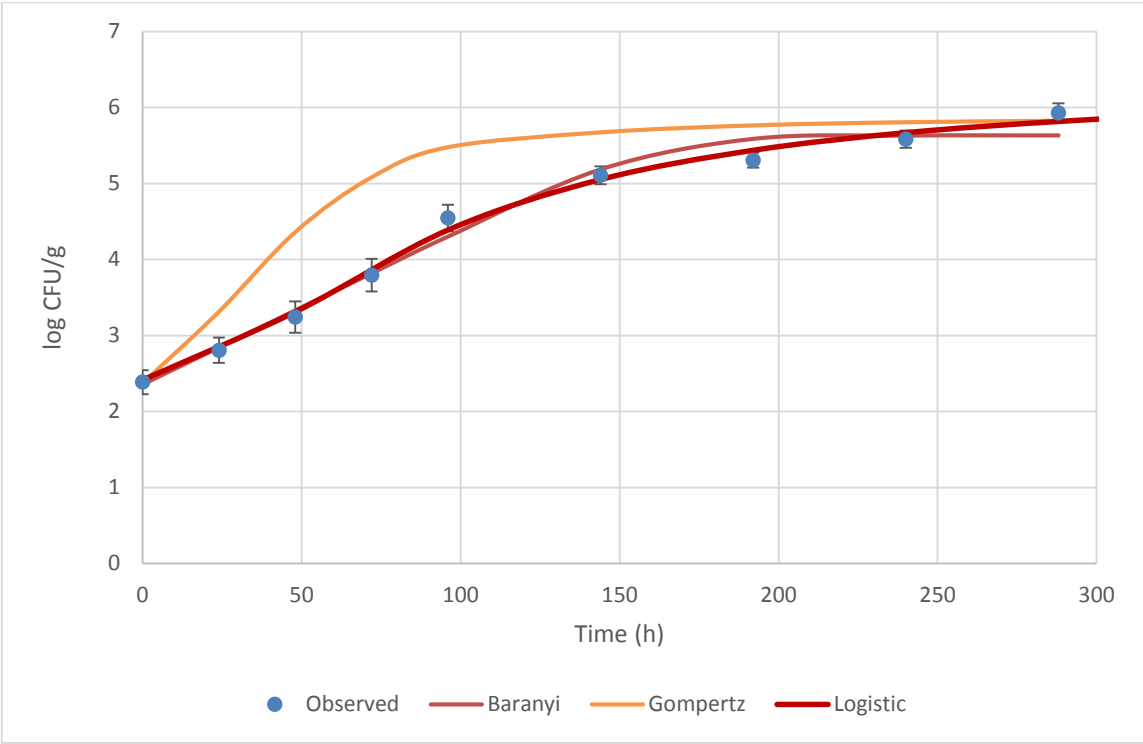


Figure A.4. The observed growth of *Listeria innocua* on fresh-cut cantaloupe at 10°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

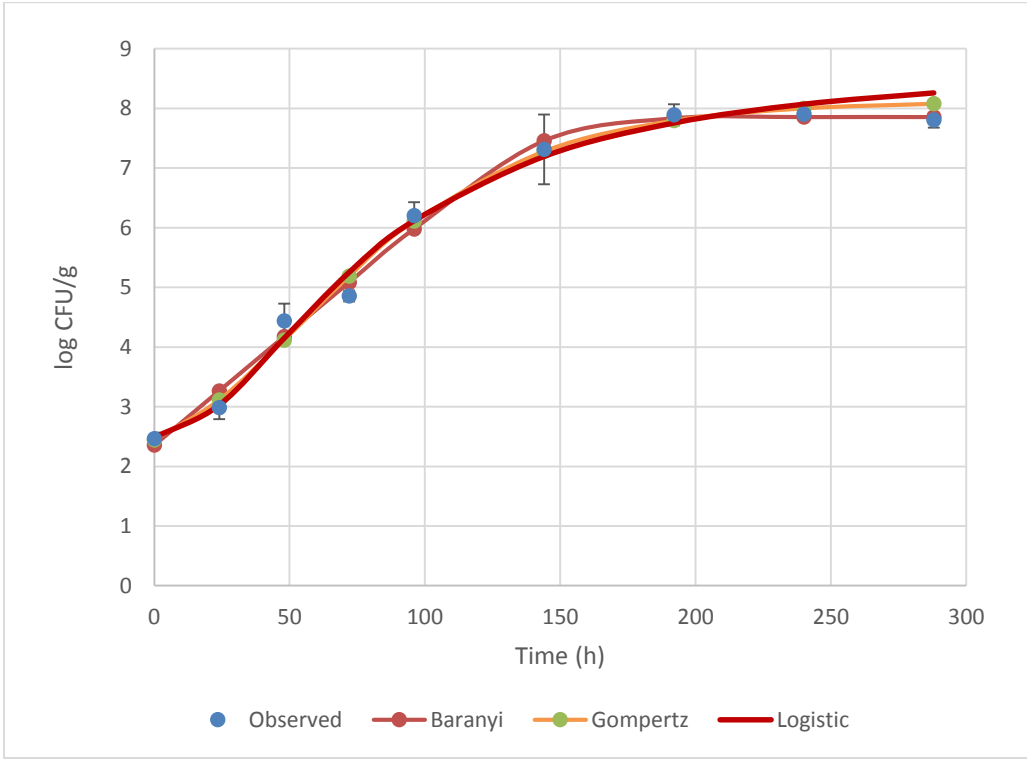


Figure A.5. The observed growth of *Listeria innocua* on fresh-cut romaine lettuce at 25°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

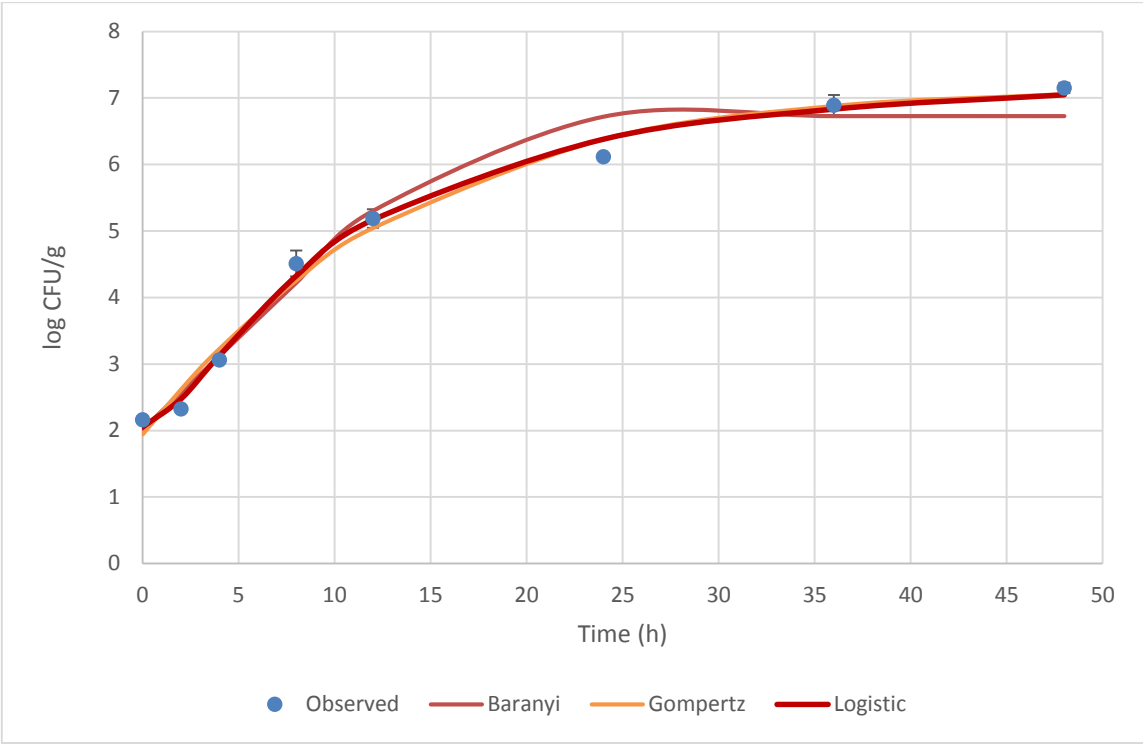


Figure A.6. The observed growth of *Listeria innocua* on fresh-cut cantaloupe at 25°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

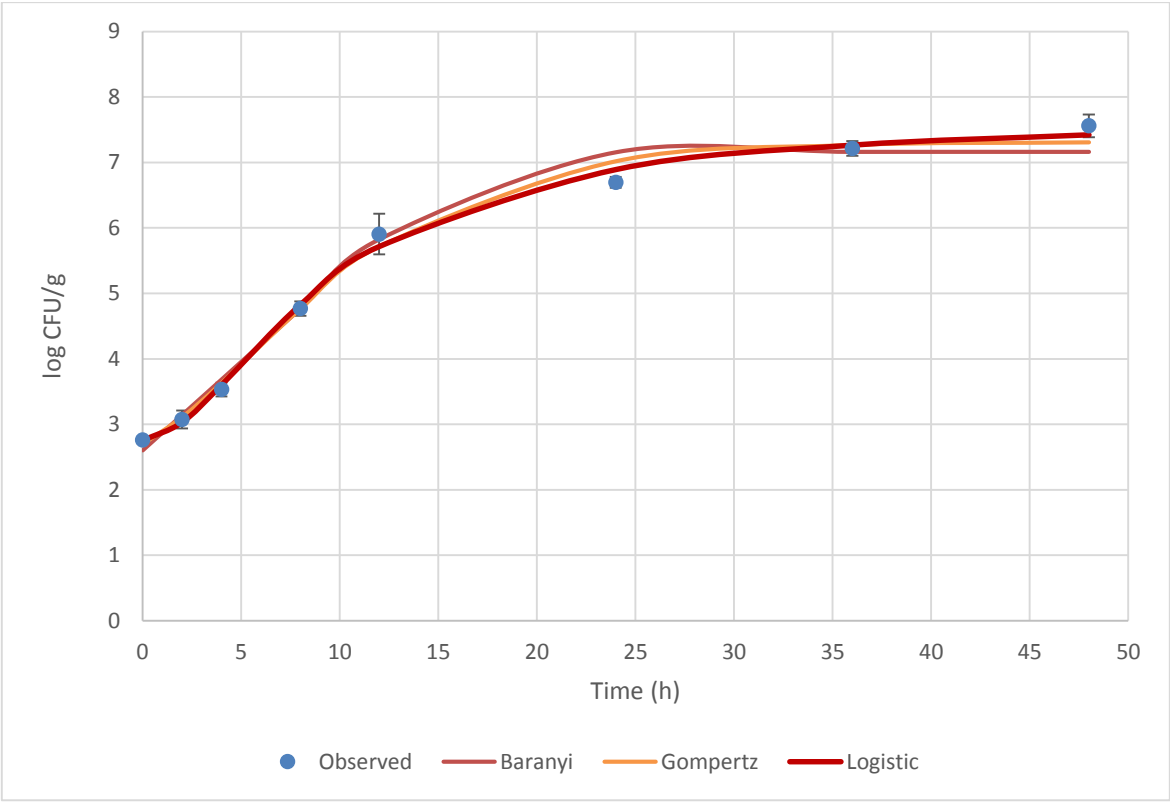


Figure A.7. The observed growth of *Listeria innocua* on fresh-cut romaine lettuce at 30°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

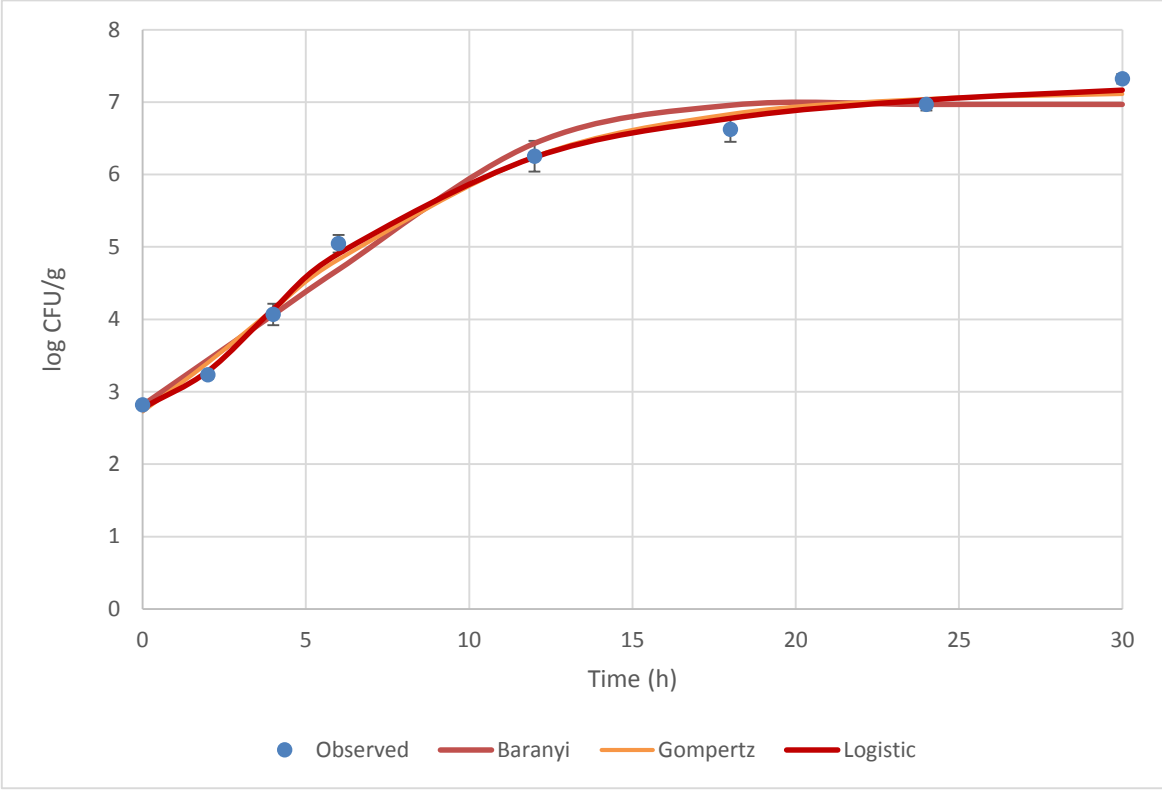


Figure A.8. The observed growth of *Listeria innocua* on fresh-cut cantaloupe at 30°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

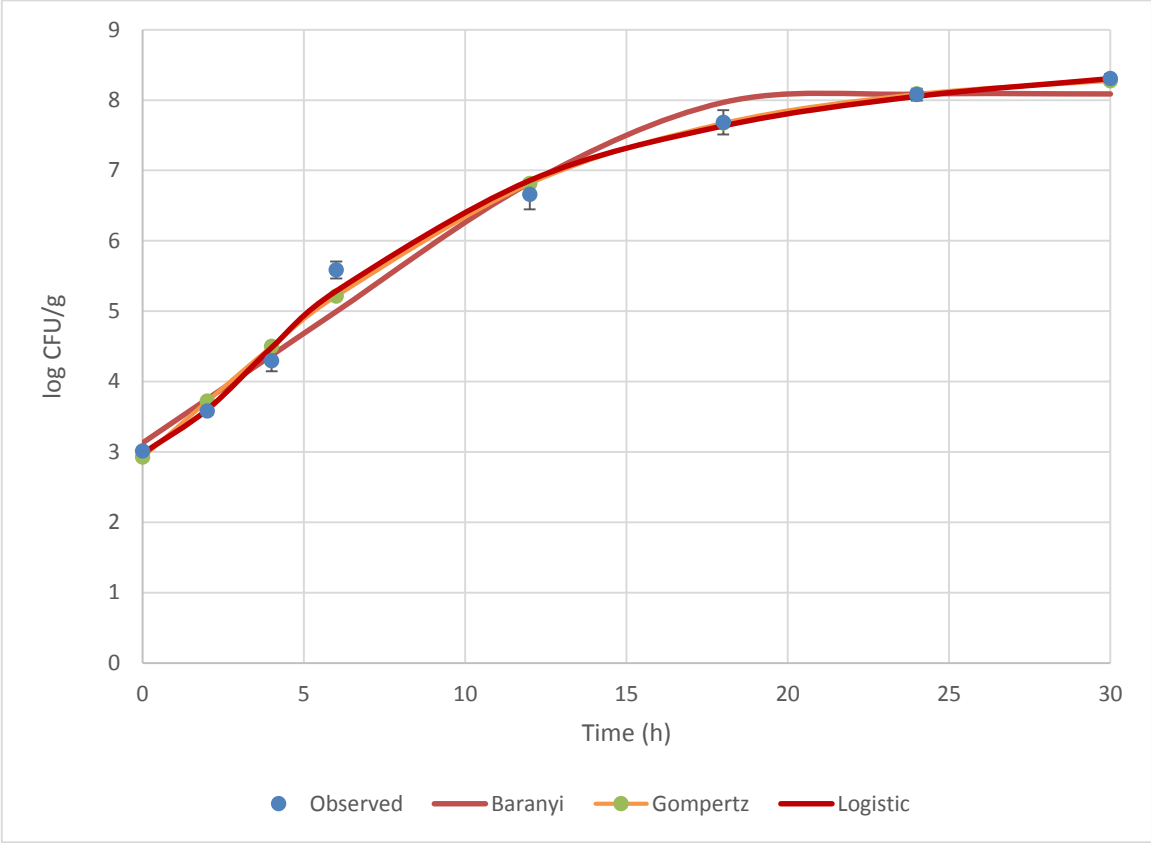


Figure A.9. The observed growth of *Listeria innocua* on fresh-cut romaine lettuce at 36°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

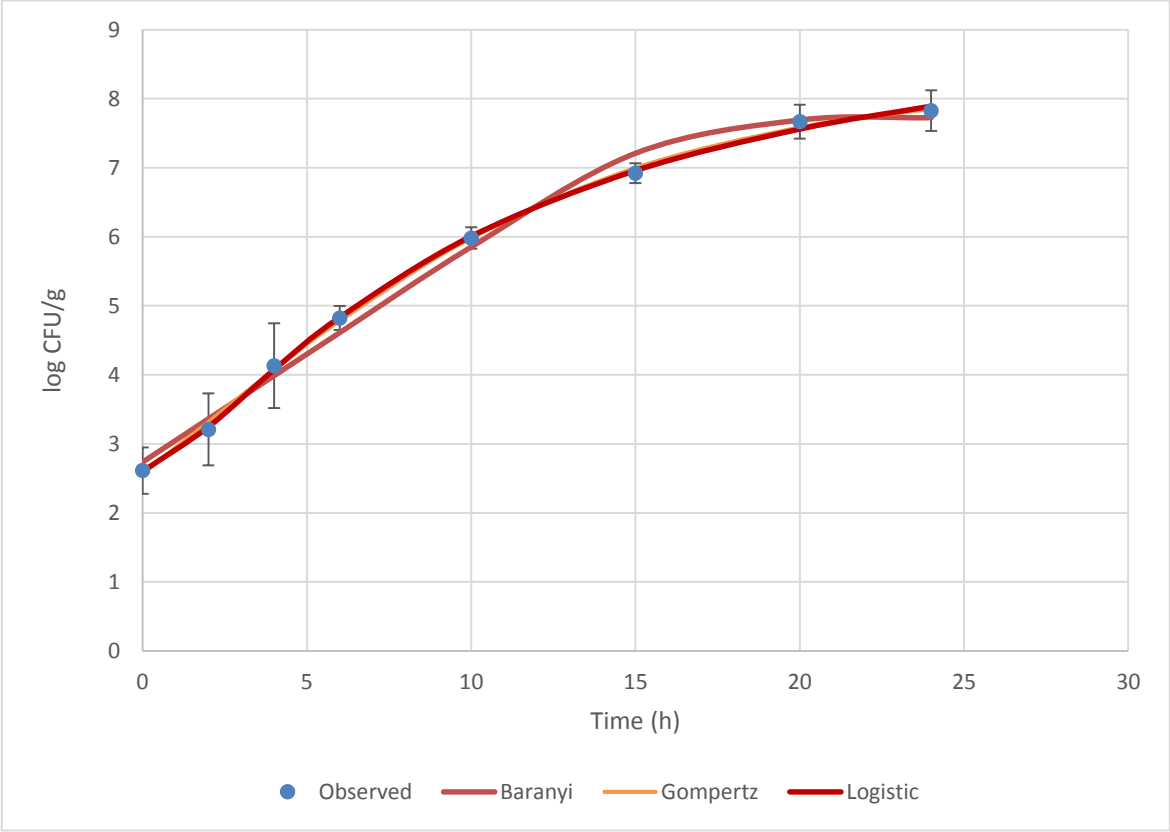


Figure A.10. The observed growth of *Listeria innocua* on fresh-cut cantaloupe at 36°C by fitting Baranyi and Roberts, Gompertz, and Logistic models (Eqs. 2.1, 2.2, and 2.4).

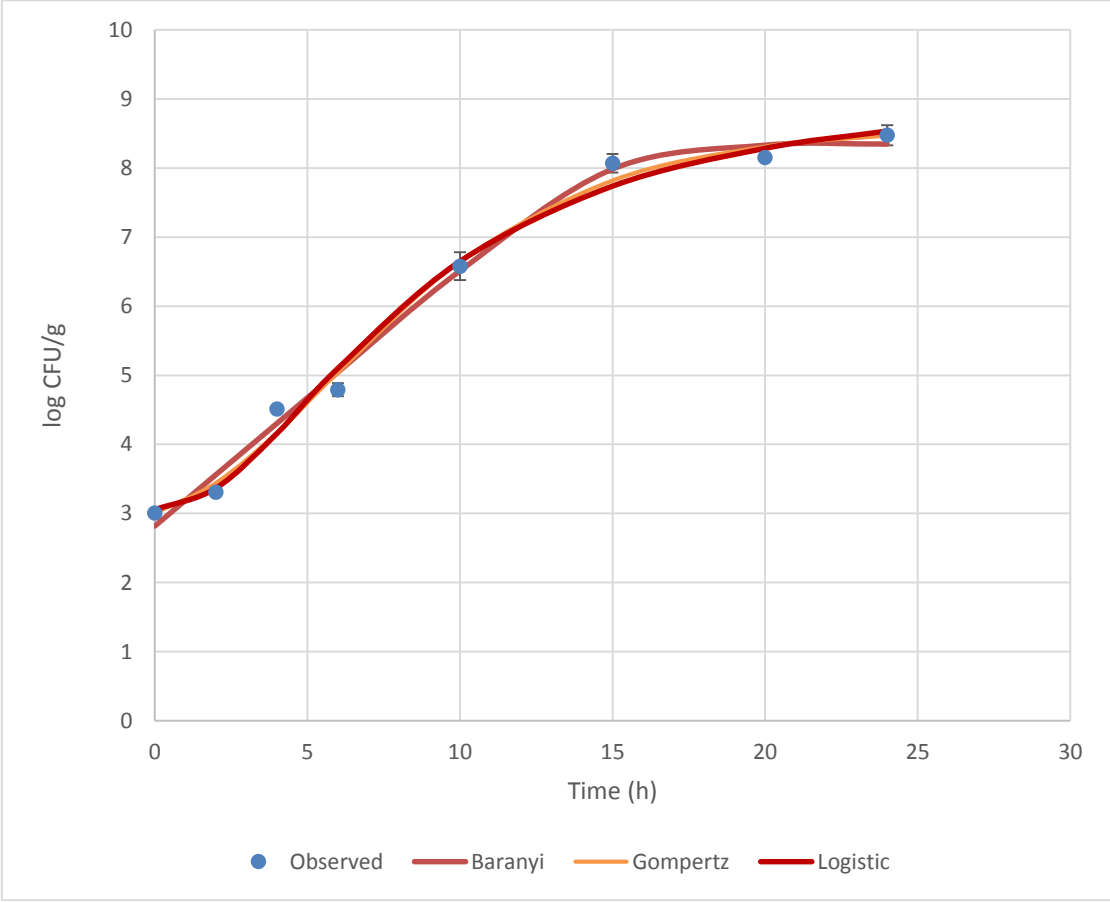


Figure A.11. The observed growth *Listeria innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe at 5°C.

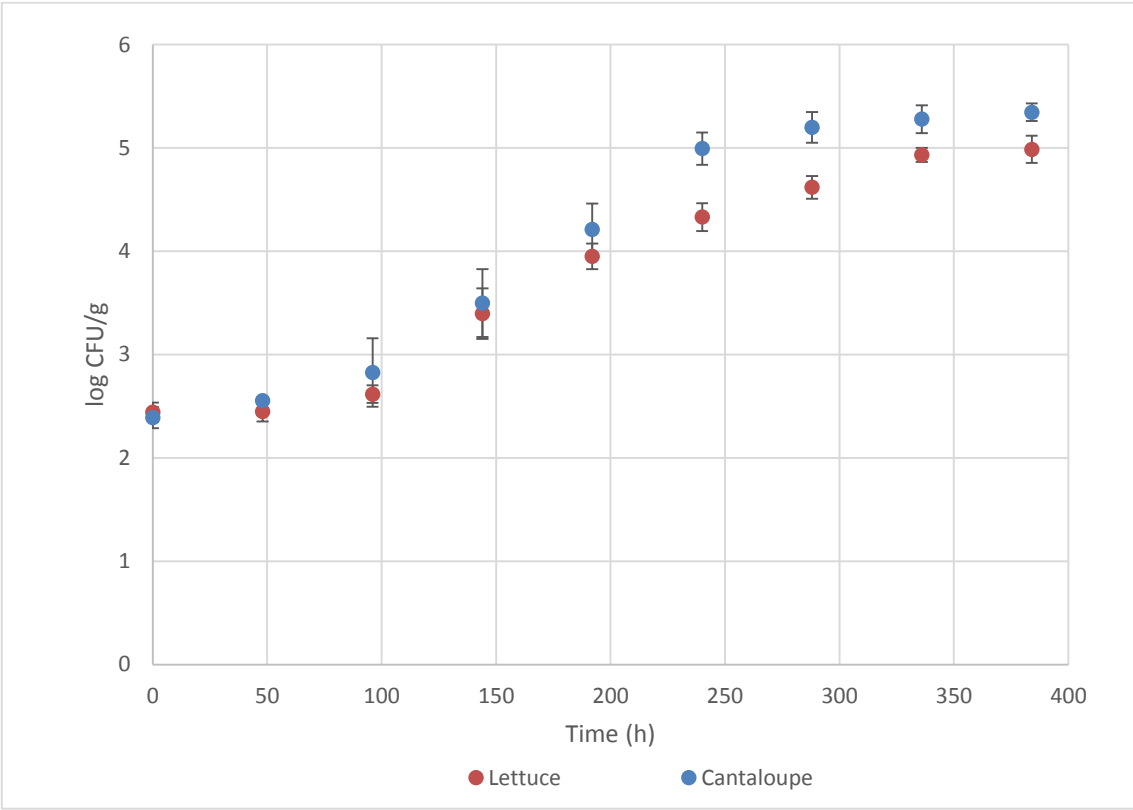


Figure A.12. The observed growth *Listeria innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe at 10°C.

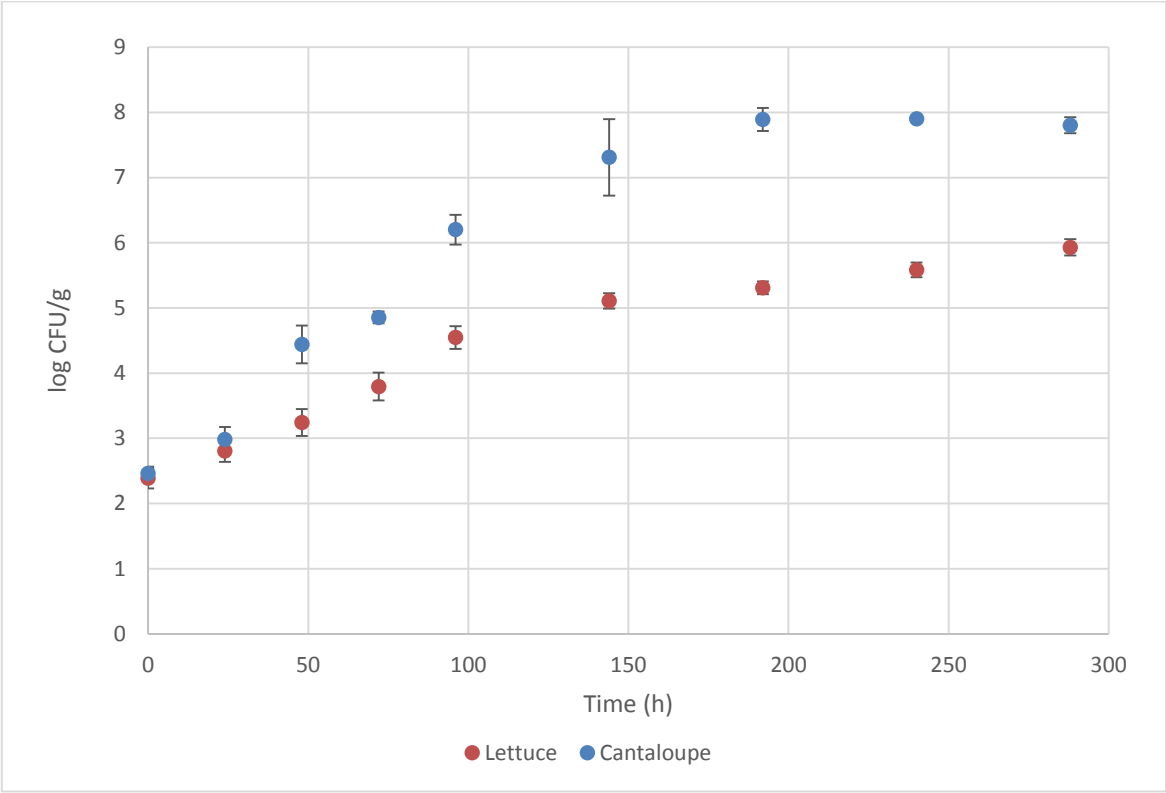


Figure A.13. The observed growth *Listeria innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe at 25°C.

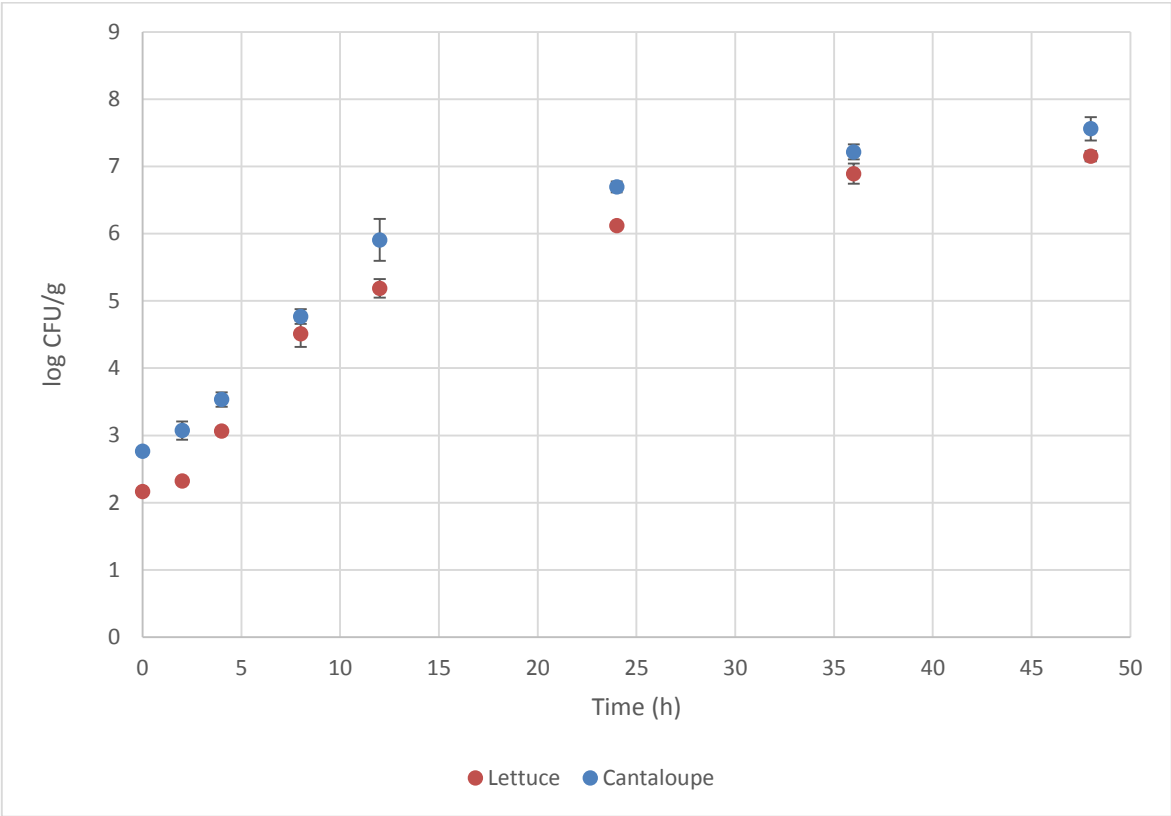


Figure A.14. The observed growth *Listeria innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe at 30°C.

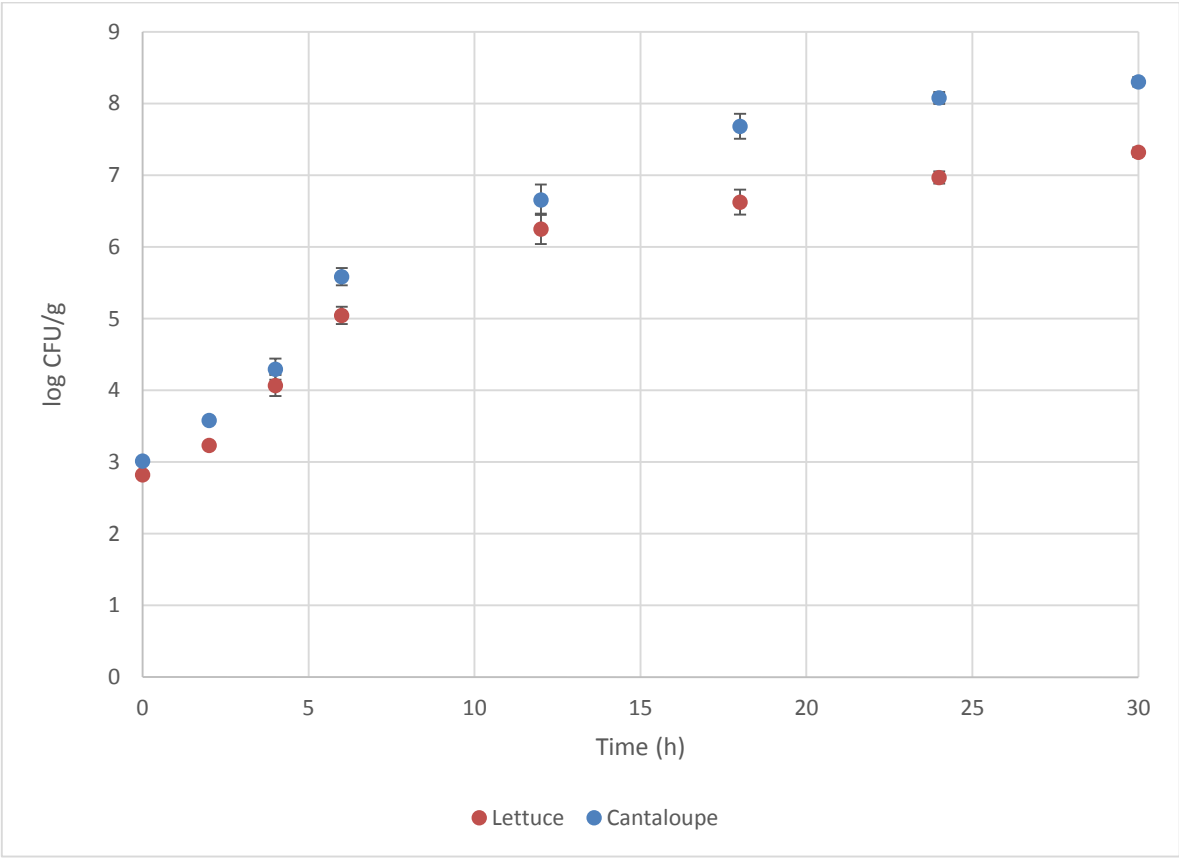


Figure A.15. The observed growth *Listeria innocua* on fresh-cut romaine lettuce and fresh-cut cantaloupe at 36°C.

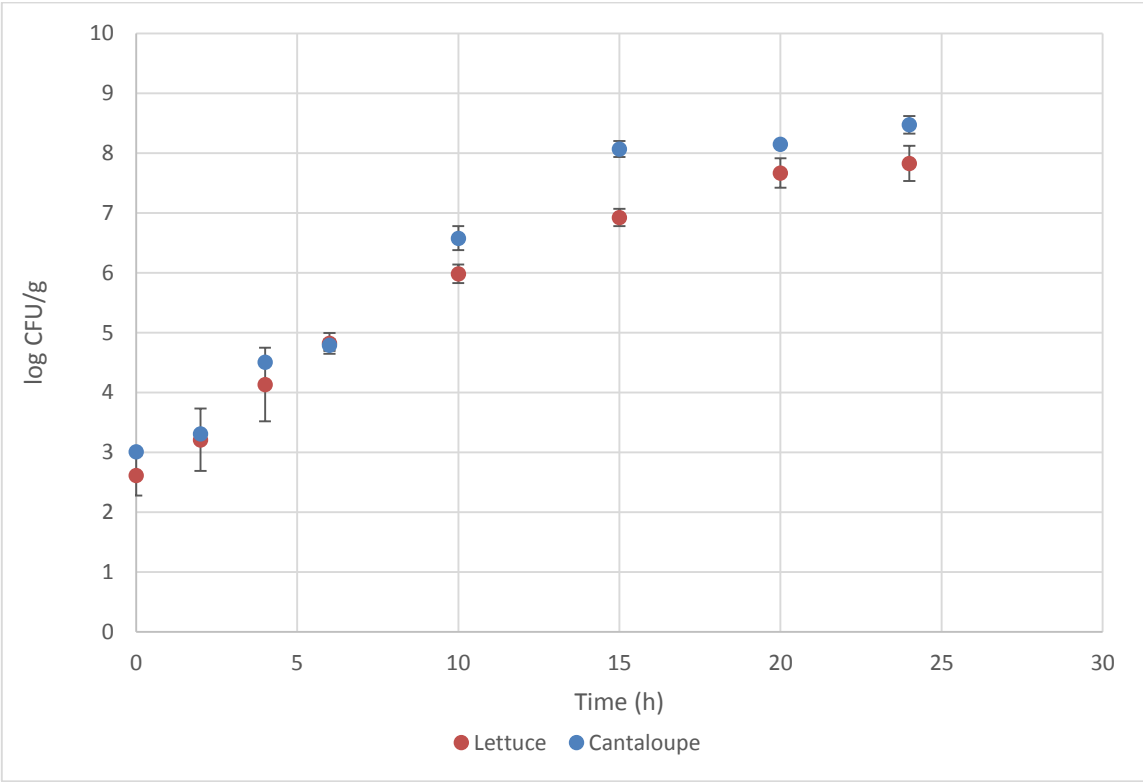
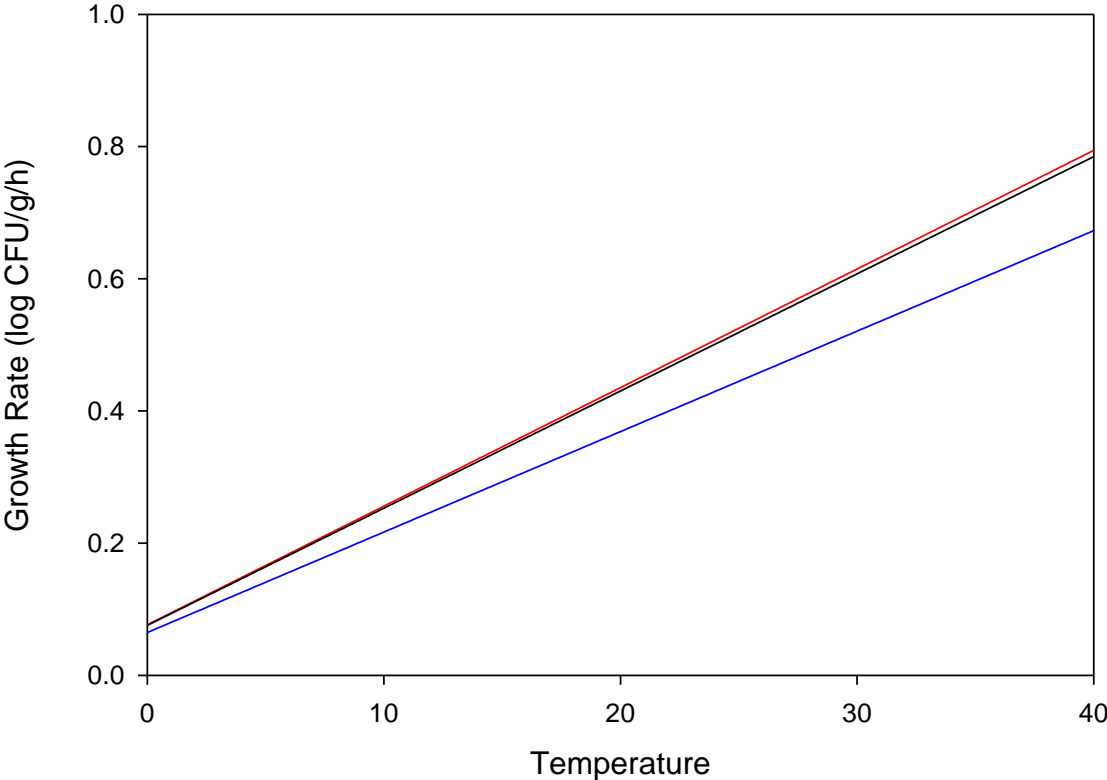
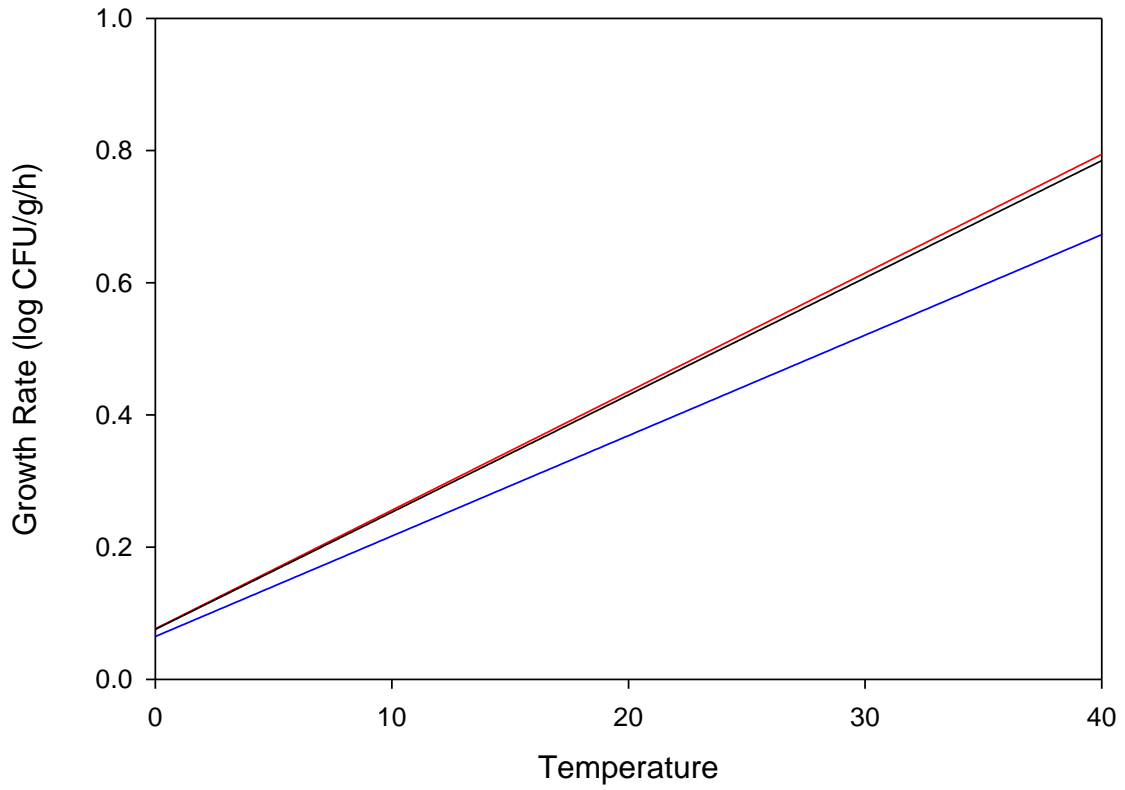


Figure A.16. Maximum growth rate of *L. innocua* on fresh-cut romaine lettuce leaves as a function of temperature.



Blue: Baranyi Model (2.4)
Black: Logistic Model (2.1)
Red: Gompertz Model (2.2)

Figure A.17. Maximum growth rate of *L. innocua* on fresh-cut cantaloupe leaves as a function of temperature.



Blue: Baranyi Model (2.4)
Black: Logistic Model (2.1)
Red: Gompertz Model (2.2)

Figure A.18. Lag time of *Listeria innocua* on fresh-cut romaine lettuce as a function of temperature.

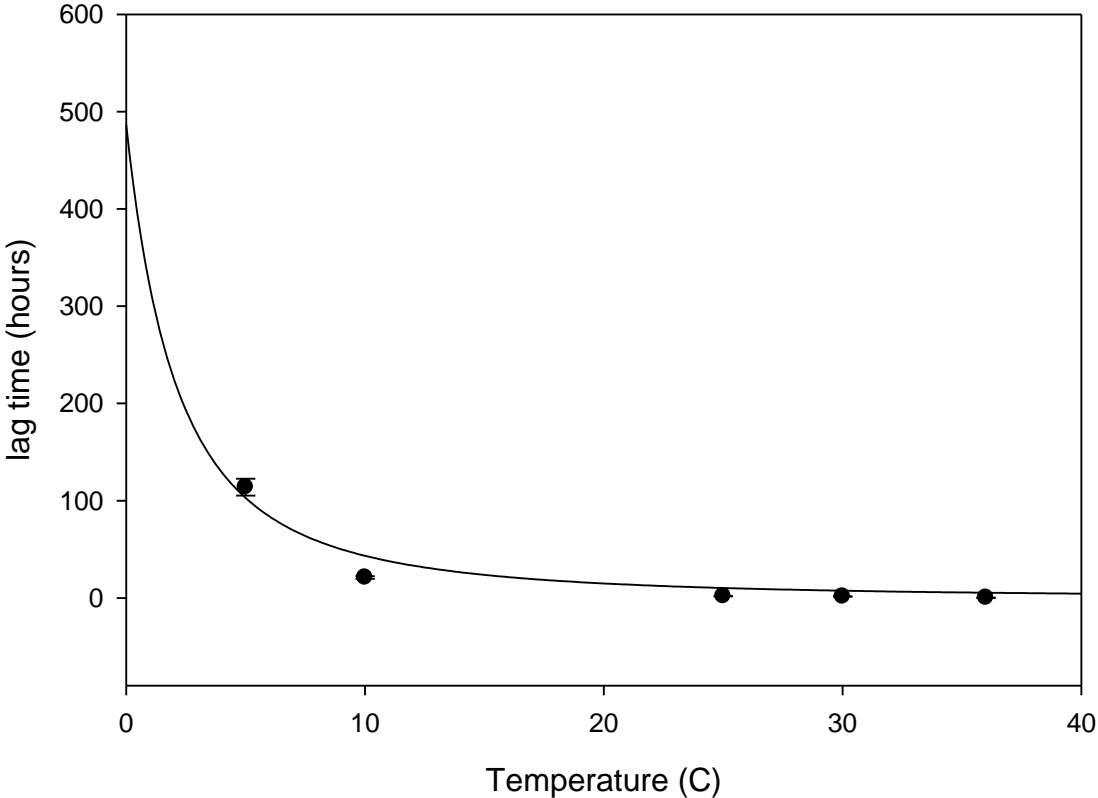


Figure A.19. Lag time for *Listeria innocua* on fresh-cut romaine lettuce as a function of temperature.

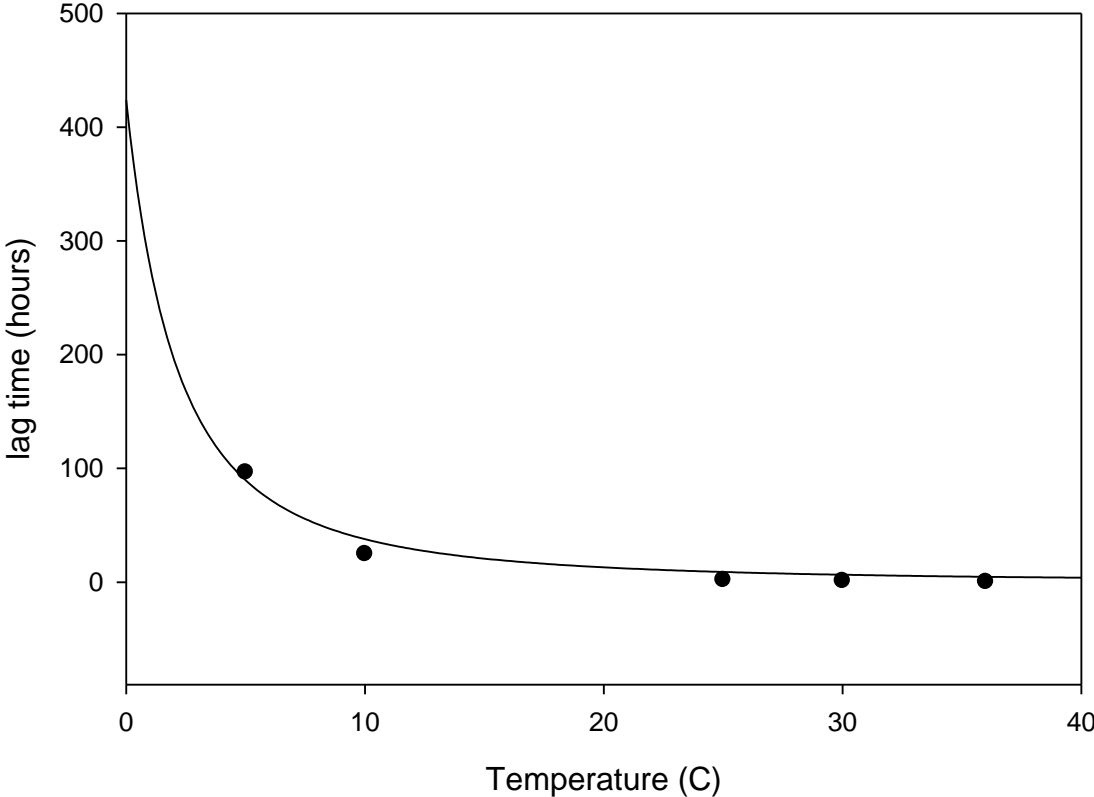


Figure A.18. Maximum population density of *Listeria innocua* on fresh-cut romaine lettuce as a function of temperature.

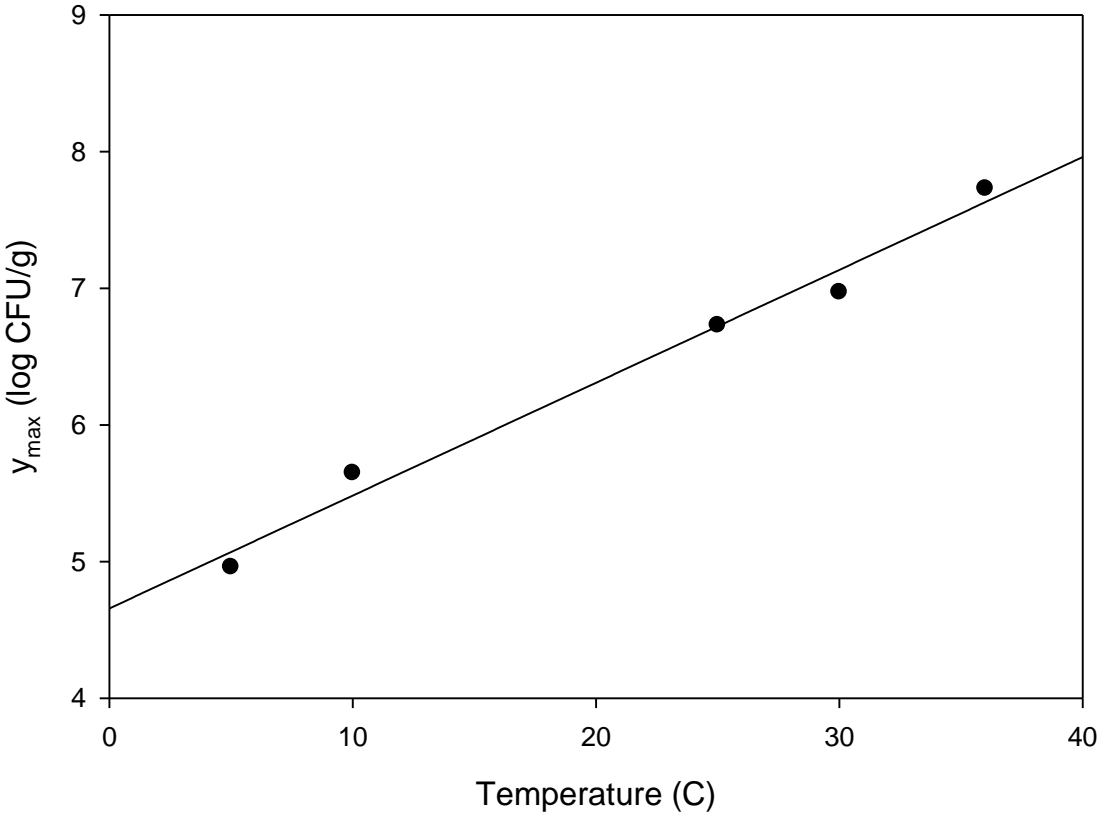


Figure A.19. Maximum population density for *Listeria innocua* on fresh-cut cantaloupe as a function of temperature.

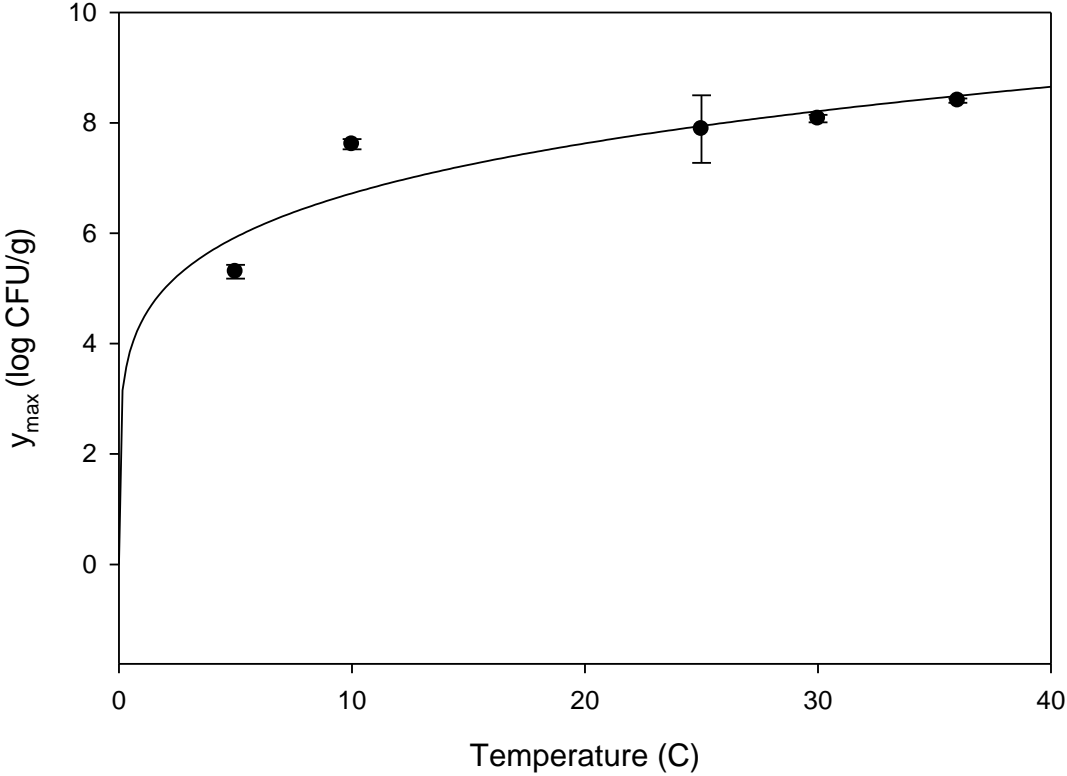


Table A.1 Overview of simulation variables and parameters for fresh-cut romaine lettuce.

Unit	Variable	Value	Source
Percent	Prevalence of contamination lettuce	0.113333333	Table 4.1
Log CFU/g	Initial Concentrations	0.3354	Table 4.2
	Washing log Reductions		
Log CFU/g	Water	0.5845	Experimental
Log CFU/g	Chlorine	0.974	Experimental
Log CFU/g	Cross Contamination	-1.2	
Log CFU/g	Pathogen concentration after washing treatments	-1.2231	
kGy	Irradiation Dose	1kGy	Mintier and Foley
CFU/g	Pathogen concentration after irradiation		
Log CFU/g	Pathogen concentration after disinfection treatments	-1.223100	Calculated
LN CFU/g	Pathogen concentration after disinfection treatments	-2.816292	Calculated
Transportation and Retail Storage			
Hours	Transportation time	14	Jacxsens et al 2002
°C	Temp, retail	3.76	Nunes et al 2003
Hours	Time, retail	108	Omac et al. 2013

Hours	Total time	61	Uniform Distribution
Home storage			
°C	Temp, home, mean	4.06	Pouillot et al 2010
°C	Temp, difference from mean	2.31	Pouillot et al 2011
°C	Temp, above or below mean	1	Probability Calculations
°C	Home temp used	6.37	Probability Calculations
°C	Temperature for used	5.065	Probability Calculations
Hours	Min. time to first	27.12	Danyluk and Schaffner 2011
Hours	Max. time to first	68.16	Danyluk and Schaffner 2011
Hours	Min. time to first from farm to home	88.12	Danyluk and Schaffner 2011
Hours	Max. time to first from farm to home	129.16	Danyluk and Schaffner 2011
Hours	Time to first from farm to home	128.3302406	Danyluk and Schaffner 2011

Hours	Min. time to last	41.52	Danyluk and Schaffner 2011
Hours	Max. time to last	191.04	Danyluk and Schaffner 2011
Hours	Min. time to last from farm to home	102.52	Danyluk and Schaffner 2011
Hours	Max. time to last from farm to home	252.04	Danyluk and Schaffner 2011
Hours	Time to last	250.6444535	Danyluk and Schaffner 2011
Hours	Time to used if first is after last	0	
Hours	Time from uniform ditribution	189.487347	
Hours	Time selected for consumption	189.487347	
	Growth		
log CFU/g/hr	Growth model b parameter	0.0159	Eq 2.4
oC	Growth model Tmin parameter	-4.26	Koseki et al (2005)
1/Hours	Maximum growth rate	0.021983252	Calculated
	tlag c parameters	0.011	Model
	Lag time model Tmin parameter	-4.26	Koseki et al (2005)
Hours	tlag	95.04230244	Calculated
	F(t)	99.18752086	Calculated
	y _{max} A1 parameters	0.0826	Model

	y _{max} A2 parameters	4.6568	Model
LN CFU/g	y _{max}	11.68811421	Calculated
Log CFU/g	y _{max}	5.076083504	Calculated
LN CFU/g	y(t)	-6.358315E-01	Calculated
Log CFU/g	y(t)	-2.761381E-01	Calculated
Log CFU/g	Limit level of if >y _{max}	-0.276138132	Calculated
Serving and dose-response			
g	Serving size	48.25	Carrasco et al 2010
CFU/g	Level non Log	0.529495005	Calculated
	Level per serving	25.54813401	S.Size * Pathogen Level
	a-value	2.50E-01	Weibull Coefficient
	b-value	2.14	Weibull Coefficient
	healthy	95499258602	Weibull Coefficient
	elderly	1.8197E+15	Weibull Coefficient
	Probability of illness (healthy)	2.69E-09	
	Probability of illness (elderly)	1.41E-13	
susceptible	Probability of illness (exposure)	3.05E-10	PI*Prevalence
healthy	Probability of illness (exposure)	1.60E-14	PI*Prevalence
susceptible	Log Probability of illness	-9.52E+00	Log(PI)
healthy	Log Probability of illness	-1.38E+01	Log(PI)
	Annual Serving	22280593990	Population * S. Size

susceptible	Risk Output	6.79	PI*Annual Serving
healthy	Risk Output	3.57E-04	PI*Annual Serving

Table A.2. Overview of simulation variables and parameters for fresh-cut cantaloupe

Unit	Variable	Value	Source
Percent	Prevalence of contamination		Chen et al 2013
	lettuce	0.0655	FDA 2003
Log CFU/g	Initial Concentration	-0.97	Chen et al 2013
Washing log Reductions			
Log CFU/g	Water		N/A
Log CFU/g	Chlorine		N/A
Log CFU/g	Cross Contamination		
Log CFU/g	Pathogen concentration after washing treatments	-0.97	
kGy	Irradiation Dose		
CFU/g	Pathogen concentration after irradiation		
Log CFU/g	Pathogen concentration after disinfection treatments	-9.70E-01	
LN CFU/g	Pathogen concentration after disinfection treatments	-2.233508	
Transportation and Retail Storage			
Hours	Transportation time	14	Omac et al 2013
oC	Temp, retail	3.76	Nunes et al 2003
Hours	Time, retail	40	Omac et al. 2013

Hours	Total time	27	Uniform Distribution
	Home storage		
oC	Temp, home, mean	3.4	Chen et al 2013
oC	Temp, difference from mean	2.41	Chen et al 2014
oC	Temp, above or below mean	1	Danyluk and Schaffner 2011
oc	Home temp used	5.81	Danyluk and Schaffner 2011
oC	Temperature for used	4.785	Danyluk and Schaffner 2011
Hours	Min. time to first	27.5	Danyluk and Schaffner 2011
Hours	Max. time to first	68.16	Danyluk and Schaffner 2011
Hours	Min. time to first from farm to home	54.5	Danyluk and Schaffner 2011
Hours	Max. time to first from farm to home	95.16	Danyluk and Schaffner 2011
Hours	Time to first from farm to home	94.1833137	Danyluk and Schaffner 2011

Hours	Min. time to last	41.52	Danyluk and Schaffner 2011
Hours	Max. time to last	240	Danyluk and Schaffner 2011
Hours	Min. time to last from farm to home	68.52	Danyluk and Schaffner 2011
Hours	Max. time to last from farm to home	267	Danyluk and Schaffner 2011
Hours	Time to last	264.8062849	Danyluk and Schaffner 2011
Hours	Time to used if first is after last	0	Danyluk and Schaffner 2011
Hours	Time from uniform distribution	179.4947993	Danyluk and Schaffner 2011
Hours	Time selected for consumption	179.4947993	Danyluk and Schaffner 2011
Growth			
log CFU/g/hr	Growth model b parameter	0.0254	Eq. 2.6
oC	Growth model Tmin parameter	-4.26	Koseki et al (2005)
1/Hours	Maximum growth rate	0.052781846	Eq. 2.6
	tlag c parameters	0.0115	

	Lag time model Tmin parameter	-4.26	Koseki et al (2005)
Hours	tlag	92.42451428	Eq. 2.4
	F(t)	87.25914121	Eq. 2.4
	y _{max} A1 parameters	4.42	Model
	y _{max} A2 parameters	0.18	Model
LN CFU/g	y _{max}	13.49257861	Eq. 2.4
Log CFU/g	y _{max}	5.859752435	Eq 2.4
LN CFU/g	y(t)	2.372176358	
Log CFU/g	y(t)	1.030223E+00	
Log CFU/g	Limit level of if >y _{max}	1.030223E+00	
Serving and dose-response			
g	Serving size	112.5	Hoelzer et al 2012b
CFU/g	Level non Log	10.720699	Calculated
	Level per serving	1206.078637	S.Size * Pathogen Level
	a-value	0.25	Weibull Coefficient
	b-value	2.14E+00	Weibull Coefficient
	healthy	95499258602	Weibull Coefficient
	elderly	1.8197E+15	Weibull Coefficient
	Probability of illness (healthy)	1.02819E-05	PI

	Probability of illness (elderly)	5.40E-10	PI
susceptible	Probability of illness (exposure)	6.73E-07	PI*Prevalence
healthy	Probability of illness (exposure)	3.53E-11	PI*Prevalence
susceptible	Log Probability of illness	-6.17E+00	Log(PI)
healthy	Log Probability of illness	-1.05E+01	Log(PI)
	Annual Serving	3.56E+09	Population * S. Size
susceptible	Risk Output	2400.579058	PI*Annual Serving
healthy	Risk Output	1.26E-01	PI*Annual Serving

Table A.3. Estimated number cases of listeriosis associated with fresh-cut romaine lettuce consumption based on healthy and susceptible populations

Scenarios	The estimated cases of listeriosis per year	
	Healthy	Susceptible
#1	<1	<1
#2	<1	<1
#3	<1	<1
#4	<1	<1
#5	<1	<1
#6	<1	<1
#7	<1	6
#8	29	128

#1: Baseline

#2: Baseline + Irradiation

#3: Ozone

#4: Peroxyacetic acid

#5: Cold Atmospheric Plasma

#6: Baseline + Cross contamination

#7: Baseline +Temperature abuse (20°C, 24h)

#8: Baseline + Consumption time (334 hours)

Table A.3. Estimated number cases of listeriosis associated with fresh-cut cantaloupe consumption based on healthy and susceptible populations

Scenarios	The estimated cases of listeriosis per year	
	Healthy	Susceptible
#1	17	69
#2	<1	<1
#3	68	223
#4	54	220
#5	6685	31000

#1: Baseline

#2: Baseline + Irradiation

#3: Baseline + Cross contamination

#4: Baseline +Temperature abuse (20°C, 24h)

#5: Baseline + Consumption time (334 hours)